Aging in Rodents Fed Restricted Diets*†

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Section Editor's Note:

This is the first article of a new permanent section of the journal, "Geriatric Bioscience." The Editor and the Section Editor wish to use this section to present "work in progress" and/or state of the art reviews in areas of basic science of relevance to clinicians dealing with geriatric patients. We hope this can be a mechanism to help the clinician keep abreast of new information from the fields of physiology, anatomy, molecular and cell biology, neurobiology, immunology, etc. that are likely to impact upon our care of the geriatric patient now or in the future.

We wish to encourage the submission of papers for this section that will summarize recently published work from individual investigators or investigative groups and place it in the perspective of the general state of work in the field. We welcome very broad participation in this series and also solicit the readership's views on topics or work of interest that might be commissioned for this series.

Harvey Jay Cohen, MD
Section Editor

Several strategies (e.g., drugs, hormones, diet supplements and restrictions, exercise, surgery) have been tested for possible anti-aging effects in animals. These protocols are evaluated by influences on survivorship (mean and maximal survival times, mortality rates), on late-life disease patterns, and on indexes of biologic age. The most effective anti-aging strategy tested in mammals is dietary restriction (DR). Dietary restriction increases both mean and maximum survival times in diverse species including the protozoan *Tokophyra*,[1] rotifers,[2] *Daphnia*,[3] fish,[4] rats,[5–9] and mice.[10–12] Only DR convincingly decreases mortality rates[13] and increases maximum survival times[14,15] in homeotherms. Spontaneous late-life diseases occur at later ages or in lower incidences in rodents on DR.[9,16–20]

Experiments on longevity and disease incidence comprised most of the DR work from its onset in the 1930s until the mid-1970s. Now, several laboratories are investigating biologic mechanisms underlying those effects. Review of the evidence shows that old rodents on DR are biologically younger than are age-matched controls[21–24]; however, the precise way DR retards aging remains unknown.

Weaning-initiated DR (WDR) of rodents entailing a 20–60 per cent decrease in food intake starting at ~4 weeks of age is associated with 10–300 per cent increases in mean and maximum survival times.[5–10,12] The largest percentage increases were observed in rats by Ross[6] and appear to partially result from negative effects of certain diets fed ad lib. In most studies on long-lived mouse or rat strains, maximum lifespans for unrestricted animals range from 30 to 40 months vs. 40 to 50 months for rodents on WDR.

The great majority of DR studies have tested WDR and not adult-initiated DR (ADR). Only ADR seems potentially applicable to humans.

Diverse DR strategies prolong life. All limit calorie intake while apparently providing adequate amounts of other diet essentials (*under*nutrition without *mal*nutrition). We obtain this result by feeding mice limited amounts of a semipurified diet enriched in protein, vitamins, and minerals (Table 1). Over one week, mice on DR eat about the same amount of protein, vitamins, and minerals as do controls, but eat less carbohydrate, fat, and fiber. Masoro's group at the University of Texas (San Antonio) feed rats on DR a semipurified, vitamin-enriched diet at 60 percent of the daily ad lib intake.[9] Dietary restriction is carried out at the Gerontology Research Center (Baltimore) by allowing free access to a commercial chow diet but only every other day.[25] Merry and Holehan at the University of Hull (England) feed rats on DR a pelleted diet at 50 per cent of the ad lib level.[26]

Since 1976, I have studied aging in mice from long-lived strains on DR. This work has been carried out with Roy Walford. We have mainly ex-

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TABLE 1. Composition of Diets (g/kg Diet)

Constituents	Diet 1*	Diet 2†
Casein, vitamin-free	200.0	350.0
Cornstarch	260.8	157.6
Sucrose	260.8	157.6
Corn oil	135.0	135.0
Non-nutritive fiber	56.4	40.0
Salt mixture, USP XIV	60.0	110.0
Vitamin mixture	23.0	42.0
Brewers' yeast	4.0	7.4
Zinc oxide	0.05	0.1

* Diet fed to control mice. Fed as seven ~3.0-g feedings (one daily feeding on Monday through Thursday, three feedings on Friday) per week, providing ~85 kcal/week.

† Diet fed to restricted mice. Thirty-five per cent casein diet, enriched also in salt and vitamin mixtures, Brewers' yeast, and zinc oxide. Fed as four ~3.0-g feedings (one daily feeding on Monday and Wednesday, two feedings on Friday) per week, providing ~50 kcal/week.

From Weindruch R: Dietary restriction and the aging process, *in* Armstrong D, et al: Free Radicals in Molecular Biology and Aging. New York, Raven Press, 1984, p 182. Copyright 1984 by Raven Press.

amined influences of WDR and ADR on longevity, cancer incidence, and age-sensitive immunologic functions. Other effects of WDR (e.g., liver mitochondrial respiration, eye lens aging, body temperature) were also studied. We view it best to use long-lived strains in aging studies. Control mice from the strains we study have mean lifespans of ~32–38 months when fed semipurified diets; in contrast, in an oft-cited paper by Stuchlikova et al.[27], mean survival for control and WDR mice was only ~21–24 months and no mouse reached 36 months. In this review, both WDR and ADR studies in rodents are discussed concerning effects on: 1) longevity and diseases, and 2) physiology (e.g., body composition, endocrine and immune systems). Recent findings are emphasized.

Weaning-Initiated Dietary Restriction

LONGEVITY AND DISEASES

Several workers recently reported longevity and/or disease incidence data for rodents on WDR. The findings accord with previous ones. Yu et al.[9] studied male Fischer 344 rats put on DR (60 per cent of ad lib) at 6 weeks of age. The mean lifespan was 32 months (vs. 23 months for controls fed ad lib) and the maximum lifespan was 47 months (vs. 32 months for controls). Renal and other lesions occurred earlier in life in controls and progressed more rapidly. Very similar longevity was observed by Merry and Holehan,[28] who imposed WDR (50 per cent of ad lib) on male Sprague-Dawley rats. Two WDR studies from the Gerontology Research Center were reported. In one, male Wistar rats were fed ad lib or subjected to WDR.[25] Mean lifespan was higher for WDR rats (32 vs. 17 months) as was maximum lifespan (41 vs. 23 months). In the other study, mean lifespan was again higher for male Wistar rats on WDR than for ad lib controls (29 vs. 24 months) and so was maximum survival (37 vs. 30 months).[29] Everitt and colleagues studied effects of hypophysectomy and DR on aging (longevity, diseases, collagen) in male Wistar rats fed a commercial chow diet. Hypophysectomized rats eat only ~40 per cent the amount of food as do intact rats. Thus, the DR group served as pair-fed, intact controls. Young (70 day old) rats subjected to either DR or hypophysectomy outlived ad lib controls (mean survival of 28–30 months for treated rats vs. 26 months for controls; maximum survival of 42–44 months for treated vs. 37 months for controls).[30] Both treatments inhibited aging of tail collagen and late-life diseases (tumors, renal, heart enlargement, hind limb paralysis). In another study, rats were fed either 12.5, 25, 50, or 75 kcal/day.[31] Longevity was greatest for rats eating 25 or 50 kcal/day. No rat lived beyond ~41 months.

Two longevity studies involving mice on WDR were reported by Kay Cheney and others from our laboratory. C57BL/6J mice on WDR showed a greater maximum lifespan (but not mean lifespan) and lower incidence of lymphoma than did controls.[32] A semipurified diet was used with controls eating ~105 kcal/week and mice on WDR eating ~60 kcal/week. Mice on WDR eating a salt- and choline-supplemented diet lived the longest of all tested. In the other study, (C57BL/10Sn × C3H/HeDiSn)F_1 female mice (abbreviated as B10C3F_1) were fed semipurified diets similar to those of the preceding study except that all mice on DR ate a diet doubled in salt and vitamin content.[12] Preweaning DR was also tested by suckling nine pups per mother (vs. five per mother in the control group) and separating the mothers from the litter every other day starting at one week of age. These DR mice showed a median lifespan of ~45 months (vs. ~38 months for controls) and a maximum of 10 per cent survival time of ~50 months (vs. ~43 months for controls).

We studied longevity and cancer incidence in female (C3H.SW/Sn × C57BL10.RIII/Sn)F_1 mice (abbr. C3B10RF_1) put on DR at or before the age of weaning. Preliminary results were communicated[33] and a full report is being prepared. Six diet groups (n = 49–71) were set up at weaning: *Group 1*—fed Purina Lab Chow ad lib; *Group 2*—fed Diet 1 as described in Table 1 (~85

kcal/week); *Group 3*—fed Diet 2 as described in Table 1 (~50 kcal/week); *Group 4*—as per Group 3, but also restricted pre-weaning by separating pups from mother every other day one week after birth; *Group 5*—fed ~50 kcal/week but gradually restricted in protein intake (35 per cent casein diet from weaning → 4 months, 25 per cent casein from 4 → 12 months, 20 per cent from 12 → 24 months, 15 per cent from 24 months → death); *Group 6*—fed ~40 kcal/week of Diet 2. Group 2 mice probably ate less food than the ad lib amount since their adult body weights averaged ~35–40 g vs. ~45–50 g for Group 1 (and ~20–25 g for DR mice). Mean lifespans ranked: Group 1 (27.4 ± 0.9 [SE] months) < Group 2 (32.7 ± 0.7) < Group 5 (39.7 ± 0.9) < Groups 3 and 4 (42.3–42.9 ± 0.9) and Group 6 (45.1 ± 0.9). The maximum lifespans (mean lifespan for the longest-lived 10 per cent in each group) were: 35.1 ± 0.3 months for Group 1, 39.7 ± 0.6 months for Group 2, 48.5 ± 0.5 months for Group 5, 50.5–51.1 ± 0.2 months for Groups 3 and 4, and 53.0 ± 0.3 months for Group 6. To my knowledge, no prior report describes laboratory mice living as long as did the mice in Group 6. These results indicate: 1) As the severity of WDR increased, so did longevity. 2) Pre-weaning DR did not boost longevity for mice put on WDR. 3) Longevity for mice fed Purina Lab Chow ad lib was ~15 per cent less than those for mice fed a semi-purified diet in slightly less than ad lib amounts. 4) A gradual restriction of protein intake of mice on WDR led to a shorter lifespan (~6 per cent) than for mice on WDR eating a high-protein diet.

Tumor incidence was lowest for Group 6 (38 per cent of mice were tumor-bearing) and highest for Group 2 (79 per cent). Lymphoma was the most common tumor and was most frequent in Groups 1 (29 per cent incidence) and 2 (44 per cent). Mice on the high-protein DR showed less lymphoma (13–23 per cent incidence for Groups 3, 4, and 6). The mean survival for lymphoma-bearing mice was greatest for those on DR (~40 months vs. ~30 months for Groups 1 and 2). Hepatoma was the next most common tumor and the incidence was not affected by WDR; however, mean longevity for mice with hepatoma was greater in all DR groups (41–45 months) than in control groups (29–34 months).

PHYSIOLOGY

Masoro's group studied several physiologic indexes of aging in male Fischer 344 rats on WDR. Rats on WDR ate more calories per gram of body weight during their lifetimes (134 kcal/g lifetime) than did controls (92 kcal/g lifetime) suggesting that WDR does not slow aging by slowing metabolic rate.[34] Body mass, lean body mass, and adipose mass all fell in late life and each decline was greatest in controls.[9,35] Gastrocnemius muscle mass began to drop at ~18 months in controls vs. ~24 months in DR rats.[9] DR delayed age-related increases in serum levels of cholesterol and phospholipids and age-related decreases in post-absorptive serum free fatty acid concentration.[36] DR from 6 months of age was as effective as DR from 6 weeks of age in modulating age changes in serum lipids.[37] Losses with age in tension development of vascular smooth muscle were retarded by DR[38] as were losses in adipocyte responses to the lipolytic hormones glucagon and epinephrine.[39,40] Dietary restriction may prevent losses in glucagon response via effects on receptor–plasma membrane events.[41] Dietary restriction prevents senile bone loss and suppresses age-related increases in serum levels of parathyroid hormone.[42] Dietary restriction started at 6 weeks or 6 months of age inhibits age-related rises in calcitonin levels in blood and thyroid.[43]

Merry and Holehan studied puberty onset, reproductive lifespan, and hormone levels in female[26] and male[28] Sprague-Dawley rats subjected to WDR. Puberty onset in females was delayed (but not prevented) by WDR. Vaginal opening and the first estrus occurred in 75 per cent of control rats between 36 and 45 days of age but in only ~50 per cent of the females on DR by 143 days of age. By 227 days of age, all DR rats were mature. Weaning-initiated dietary restriction increased reproductive lifespan in females as ~80 per cent of the rats on WDR could conceive and wean pups at 510 days, which is 100 days *beyond* when breeding ends for controls. About one of four females on WDR could breed at 840–930 days! In males, puberty onset (judged by a peak in serum testosterone levels) occurred at ~60 days of age in controls and was delayed 10–20 days by WDR. Peak levels of testosterone were approximately threefold lower for males on WDR. Serum follicle-stimulating hormone (FSH) levels were very low in WDR males between 30 and 70 days but major effects of DR did not persist. Reproductive lifespan for males was not increased by WDR.

Wistar rats on WDR at the Gerontology Research Center showed a retarded loss with age of brain striatal dopamine receptors.[44] Levels in 24-month-old rats on WDR were ~50 per cent higher than in age-matched controls and were comparable to levels in 3- to 6-month-old controls.

Workers in India report[45] that WDR (50 per cent of ad lib intake) imposed on Swiss albino female mice reduces: 1) *in vitro* lipid peroxidation in liver homogenates, 2) lipofuscin accumulation in brain and heart and, 3) the percentage of lysosomal enzyme activity that is free (i.e., not inside the lysosome) in liver, brain, and intestinal homogenates.

Mice were studied for up to 12 months of age. Low-protein diets (6 or 12 per cent protein) fed ad lib for up to 12 months produced similar effects.[46] Survival was not reported in either study. These results were viewed as supporting the free radical theory of aging as restricted mice (which presumably would live longer) showed less signs of free radical–mediated damage.

Liver microsomal drug metabolizing and NADPH-generating enzyme activities were increased in male Sprague-Dawley rats on WDR (50 per cent of ad lib) for seven weeks.[47,48] Activities of NADPH-generating enzymes (malic enzyme, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase) were approximately two times higher in liver and three to five times higher in adipose tissue of rats on WDR as compared with controls. Liver drug metabolizing enzyme activites were ~20–50 per cent higher in rats on WDR.

Dehydroepiandrosterone (DHEA, a steroid hormone reported to have anticancer and anti-obesity effects when fed to rodents[49–51] also raises the activity of liver malic enzyme in rats.[52,53] Mice in our colony fed a diet with DHEA ate ~30 per cent less food than did controls.[54] Perhaps a decreased food intake may contribute to certain biologic effects attributed to feeding DHEA.

Eve and Gerald Reaven and colleagues studied pancreas structure and function, insulin action, and serum triglyceride levels in male Sprague-Dawley rats on WDR (as carried out by combining laboratory chow and cellulose in a 1:2 ratio and feeding ad lib). Controls ate the standard chow ad lib. At 12 months of age, rats on WDR and another group fed the control diet ad lib but allowed to exercise on a running wheel showed threefold lower serum levels of tiglycerides and insulin compared with sedentary rats on a control diet.[55] Pancreatic islets from 12-month-old sedentary controls appeared enlarged, multi-lobulated, and fibrotic; in contrast, WDR or exercised rats of this age did not show pancreas pathology.[56] Compared with 12-month-old rats fed ad lib, WDR rats showed lower plasma insulin levels after an oral glucose load and less insulin resistance.[57] Glucose-stimulated insulin secretion per volume of islet fell with age but was not influenced by WDR. Similarly, in the perfused rat pancreas, aging reduced glucose-stimulated insulin secretion per islet cell mass and this loss was not influenced by WDR.[58]

Richardson proposes[59] that DR slows aging by retarding losses in protein synthesis and gives preliminary data from studies on male Fischer 344 rats to support this view.

Free radicals and mitochondria may play a major role in the aging process.[60,61] Mitochondria produce free radicals as a result of oxidative metabolism and these radicals can damage cellular and extracellular molecules. Much evidence exists for a loss of mitochondria in old mammals[24] but little is known about influences of long-term DR on mitochondrial aging. We studied effects of aging or WDR on mitochondrial recovery and respiratory capacities using male C57Bl/6J mice for the aging study (old = 23–26 months, adult = 9–12 months) and female C3B10RF$_1$ mice (3–7 months) for the WDR study.[62] Old mice did not differ from adults in amounts of protein recovered from mitochondrial fractions of liver, brain, and spleen, but did show a lower cytochrome *c* oxidase activity in liver and spleen. Age reductions in *in vitro* respiration by mitochondria occurred in liver and spleen. Effects of WDR were studied in liver and brain. WDR lowered the recovery of liver mitochondrial protein and cytochrome *c* oxidase activity. Liver mitochondria from mice on WDR generally showed increased state 3 respiration rates (i.e., in the presence of ADP) without 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 (state 3/state 4) for the WDR group. Rates of 2,4-dinitrophenol–uncoupled respiration were also raised by WDR. Electron microscopy of liver mitochondrial preparations revealed more nonmitochondrial contaminants for old mice and larger mitochondria for mice on WDR. These findings are compatible with the notion of age-dependent losses of liver mitochondria that can be influenced by DR. A higher respiratory control index suggests better coupling of oxidative phosphorylation to electron transport. Perhaps the better coupling shown by liver mitochondria from mice on WDR results in reduced free-radical generation and less mitochondrial damage that could postpone age-related losses of mitochondria. The number of mitochondria an organism needs may be reduced by WDR and this might boost a restricted animal's capacity to make new mitochondria in late life.

With colleagues at UCLA's Jules Stein Eye Institute, we studied aging of the eye lens in C3B10RF$_1$ mice on WDR. With aging and cataract development in rodents and humans a loss occurs in the amount of soluble protein in the gamma crystallin fraction. We found that WDR retards this age-related loss in gamma crystallins[63] (Fig. 1). We are unaware of other reports that describe a deceleration of this loss.

A physiologic alteration that occurs with aging involves the immune system. Lower immune responses capacities to exogenous stimuli (e.g., T-lymphocyte mitogens, tumors, viruses) occur with

advancing age along with increases in autoimmunity.[64,65] Immunologic aging may contribute to the pathogenesis of aging.[66-67] Thymic involution may play a major role in immune senescence since involution is associated with a decline in serum thymic hormone levels,[68,69] which presumably leads to losses with age in T-cell differentiation capacities.[70] Also, aging lowers T-cell production of and response to interleukin 2 (a T cell–derived immunoregulatory factor).[71,72]

Our studies indicate that immunologic aging is influenced by WDR. Young B10C3F$_1$ females (less than 8 months old when studied) on WDR showed lower spleen weights, a dampening of thymus growth, more robust T cell–proliferative responses, and lower body temperature than did age-matched controls.[73] Similar findings were reported by Cheney et al.[12] in the same F$_1$ hybrid on a different WDR regimen. Histologic study of thymus structure in B10C3F$_1$ mice on WDR revealed a "younger" appearance of thymuses from 6-month-old WDR mice than for age-matched controls.[74] In other experiments, C3B10RF$_1$ mice were put on WDR and studied at between 3 and 15 months of age for splenic T-cell proliferation induced by mitogens.[75] A higher response to one such mitogen, phytohemagglutinin, for mice on WDR appeared to result from (at least in part) an increased proportion of responsive T cells. Natural killer (NK) cell activity was also studied in spleen cells from mice of this strain on WDR.[76] It has been suggested that NK cells defend against cancer.[77] Lower basal NK responses were seen for mice on WDR (2–33 months old) than for age-matched controls; however, after injection with polyinosinic:polycytidylic acid (which raises interferon levels and NK activity), old mice on WDR responded higher than did old, injected controls. Also mice on WDR showed higher *in vitro* generation of cytotoxic T lymphocytes to allogeneic tumors than did age-matched controls. Mice on WDR may better resist cancer via an NK system very responsive to induction signals along with higher levels of T-cell cytolysis.

Adult-Initiated Dietary Restriction

Compared to WDR, ADR has been the subject of far fewer gerontologic studies. In 1976, we became interested in ADR because the available findings did not answer the question of whether or not it was an effective anti-aging strategy. Longevity data were inconclusive (see Reference 11 for discussion) and data on other age-sensitive phenomena were (and are) very limited. Another rationale for studyng ADR is that only it (and not WDR) seems potentially applicable to humans. Thus, we carried out the studies described below. Also, new findings from other labs are described.

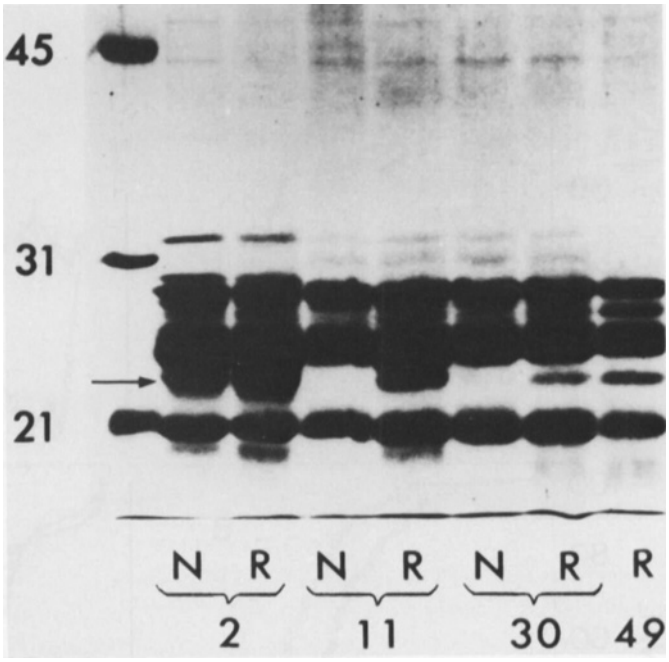

FIGURE 1. Results of sodium dodecyl sulfate–polyacrylamide gel electrophoresis of soluble proteins from lenses of diet-restricted (R) and normally fed control (N) mice aged 2, 11, and 30 months and from a diet-restricted mouse aged 49 months. The arrow identifies the gamma crystallin fraction that is lost with aging (and cataract development). This loss was decelerated by diet restriction. (From Leveille P, Weindruch R, Walford RL, et al: Science 224:1247. Copyright 1984 by the AAAS.)

LONGEVITY AND DISEASES

Male mice from two strains (B10C3F$_1$ and C57Bl/6J [abbreviated B6]) fed ad lib commercially available chow until 12–13 months of age were studied.[11] Food intake was then gradually restricted to ~60 per cent of the number of calories eaten by controls via a nutrient enriched, semi-purified diet. Controls ate a non-enriched diet in amounts to maintain body weights. Many mice were studied (B10C3F$_1$: n = 67 ADR, n = 68 control; B6: n = 29 ADR, n = 24 control). Body weights and survivorship are shown in Figure 2. The mean lifespan for B10C3F$_1$ controls was 33.0 ± 0.7 (SE) months vs. 36.9 ± 0.7 months for mice on ADR (12 per cent increase). Mean survival for the longest lived 10 per cent of each B10C3F$_1$ group (n = 7) was 40.6 ± 0.5 months for controls vs. 45.1 ± 0.6 months for ADR mice (11 per cent increase). Mean survival for B6 controls was 24.9 ± 0.9 months vs. 29.9 ± 1.4 months for ADR mice (20 per cent increase). Mean survival for the longest-lived 10 per cent of each group (n = 3) was

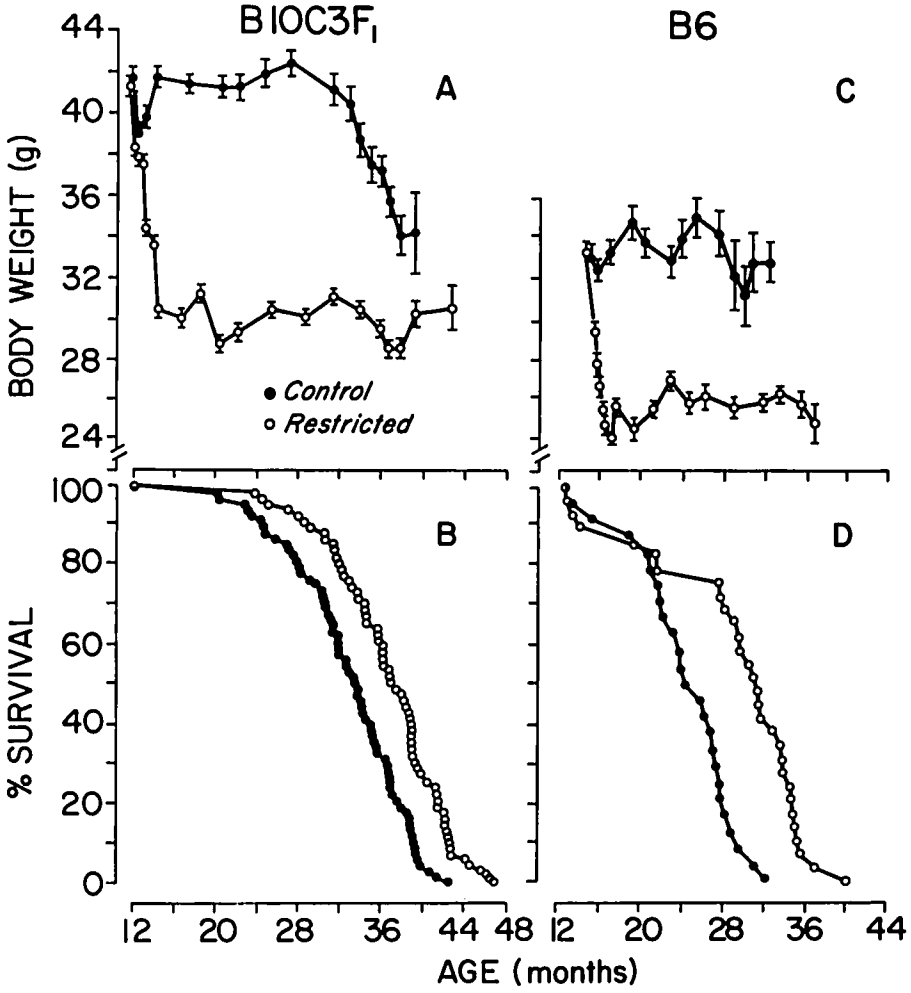

FIGURE 2. Body weights and survival of $B10C3F_1$ mice (*A* and *B*) and B6 mice (*C* and *D*) fed control or restricted diets. Each point in the survival curves represents one mouse. (From Weindruch R, Walford RL: Science 215:1415. Copyright 1982 by the AAAS.)

31.5 ± 0.5 months for controls vs. 38.2 ± 1.4 months for ADR mice (21 per cent increase). Spontaneous lymphoma incidence was lower for mice in both strains on ADR. Also, for mice bearing a lymphoma, those on ADR tended to outlive controls.

Cheney et al.[12] reported that female $B10C3F_1$ mice put on ADR at 14 months of age lived 5–10 per cent longer (average and maximum lifespans) than did controls. Mice on ADR did not live as long as WDR mice. Tumor incidence was reduced by ADR.

Goodrick and colleagues studied longevity in rodents on ADR. In one study,[78] Wistar rats were fed ad lib until 10.5 or 18 months old and then housed in either standard cages or in activity wheel cages. Part of each group was subjected to ADR (every other day feeding) and the others not restricted. ADR increased survival times in both age groups but exercise failed to yield a clear-cut effect on longevity. Maximum lifespans did not exceed 37 months. In another study,[79] DR was imposed on mice of three strains (B6, A/J, and $B6AF_1$) at either 1.5, 6, or 10 months of age. Mean lifespans were not increased in any strain by ADR from 10 months. Adult-initiated dietary restriction from 6 months increased mean lifespan in two of the three strains. Dietary restriction from 1.5 months increased lifespan in all strains.

Masoro's group reported longevity data from an ongoing study involving rats put on ADR (60 per cent of ad lib) at 6 months of age.[37] Median lifespan for these rats was ~31 months, a value intermediate to that for WDR (~35 months) and ad lib rats (~23 months).

Thus, appropriate ADR can inhibit cancer and extend average and maximum survival times.

Physiology

Very little is known about the physiology of rodents on life-prolonging ADR. A few reports describe immunologic effects and another[37] details serum hormonal and lipid levels for rats restricted as young adults (6 months old). These limited data indicate that ADR retards age changes in certain physiologic indexes.

The first immunologic findings involved mouse splenic lymphocyte proliferative responses to mi-

togens. We reported that B10C3F$_1$ male mice gradually put on ADR from 12 until 16.5 months of age showed higher responses to T-cell mitogens than did age-matched controls.[73] Similarly, Mann found that T-cell mitogen responses were increased in 22-month-old female CBA/J mice fed every other day for the preceding 5 months compared with controls fed ad lib.[80]

We next studied C3B10RF$_1$ males put on ADR at 12, 17, or 22 months of age.[81] Adult-initiated dietary restriction lowered the number of nucleated cells per spleen but increased the percentages of T cells. For mice put on ADR at 12, 17, or 22 months and tested at various ages thereafter, spleen cell proliferation after phytohemagglutinin stimulation exceeded that for age-matched controls. Splenocyte responses to concanavalin A (another T-cell mitogen) or to B-cell mitogens were not influenced by ADR. In the splenic antibody secreting cell response after injection of sheep erythrocytes, ADR and control mice differed more clearly in response kinetics than in peak levels. Splenic T-cell–mediated cytotoxicity to alloantigens fell by 20–30 per cent with age (from 5 to ~27 months) in control mice. Mice put on ADR at 12 months of age and tested at ~27 months responded like the 5-month-old controls. In another study, B10C3F$_1$ mice were put on ADR when 12 months old.[82] A small blood sample was drawn at 13 and 23 months of age and analyzed for IgG and immune complex levels. Both measures fell after ADR.

Concluding Comments

Before about 1975, aging studies on rodents subjected to dietary restriction largely examined longevity or disease incidence. More recently, DR studies have turned increasingly mechanistic and have provided endocrinologic, immunologic, and biochemical insights. Yet, the precise way that DR influences aging remains unknown. This most likely is a result of the multitude of changes brought on by DR as well as the mysterious nature of biologic aging. Dietary restriction studies are yielding a clearer understanding of biologic changes associated with lifespan extension and may well provide clues for ways to optimize the human diet.

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