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# The Retardation of Aging by Caloric Restriction: Studies in Rodents and Primates*

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ABSTRACT

Caloric restriction (CR), which has been investigated by gerontologists for more than 60 yr, provides the only intervention tested to date in mammals (typically mice and rats) that repeatedly and strongly increases maximum life span while retarding the appearance of age-associated pathologic and biologic changes. Although the large majority of rodent studies have initiated CR early in life (1–3 mo of age), CR started in midadulthood (at 12 mo) also extends maximum life span in mice. Two main questions now face gerontologists investigating CR. By what mechanisms does CR retard aging and disease processes in rodents? There is evidence to suggest that age-associated increases in oxidative damage may represent a primary aging process that is attenuated by CR. Will CR exert similar actions in primates? Studies in rhesus monkeys subjected to CR and limited human epidemiological data support the notion of human translatability. However, no matter what the answers are to these questions, the prolongation of the health span and life span of rodents by CR has major implications for many disciplines, including toxicologic pathology, and raises important questions about the desirability of *ad libitum* feeding.

*Keywords.* Longevity; nutrition; oxidative stress; mitochondria; rhesus monkey; *ad libitum* feeding

## INTRODUCTION

A recurrent theme in gerontologic research is the search for interventions that can increase species-specific maximum life span by opposing the development of age-associated diseases and biological changes. To date, only 1 intervention, caloric restriction (CR), has been shown to consistently and reproducibly yield these outcomes in mammals (with mice and rats being the usual models). The CR paradigm has been the topic of 5 books (1, 3, 11, 24, 26). Life-span extension by CR has also been observed in fish, spiders, *Daphnia* (water-flea), and other nonrodent species, which suggests a broad relationship between caloric intake and aging. The CR paradigm has assumed widespread use in gerontology, as it provides the best model available to study the biology of decelerated aging in mammals. There is also great interest in determining whether or not such actions will be obtained in primates subjected to CR (20).

## CR IN RODENTS

### *CR Initiated Early in Life*

The life span–extending actions of CR depend specifically on calorie restriction per se (9, 24). Figure 1 illustrates a clear, direct relationship between the extent of CR and the amount that life span is increased in female mice from a long-lived $F_1$ hybrid strain (25). In this study, mice on CR were fed diets enriched in protein, vitamins, and minerals so as to produce undernutrition without malnutrition.

The most common level of CR investigated is ~40% less than the average unrestricted food intake. Not surprisingly, this degree of CR produces mice and rats of very different size and body composition than their control-fed counterparts. Importantly, and as demonstrated in Fig. 1, even mild CR (~20%) extends life beyond that of *ad libitum*–fed controls. The strong inverse relationship between energy intake and longevity has led me to favor mechanisms for CR that have strong links to energy metabolism.

As discussed elsewhere (19), despite similar percentage reductions below the *ad libitum* intake in rat and mouse CR studies, it appears that the physiological severity of CR as indicated by the reduction of body temperature or blood glucose levels appears more pronounced in mice than in rats. Besides species differences (a rat is certainly not a mouse), 2 reasonable explanations for this mouse/rat differential response to CR are as follows. (a) A decreased energy expenditure would be expected for rats due to cage size–induced limitations in a rat's ability to move around its cage. In contrast, an individually housed mouse has ample room for active movement. (b) A related factor is the likely existence of greater obesity in conventionally fed rats from several of the commonly used strains (Sprague-Dawley, Wistar, Fischer-344) than in mice from most strains. A reduced physiological severity of CR in most rat studies also would accord with the fact that the very longest lived CR rodents are mice (not rats), despite the fact that the survival of *ad libitum*–fed mice and rats from most of the long-lived strains used in aging research do not differ

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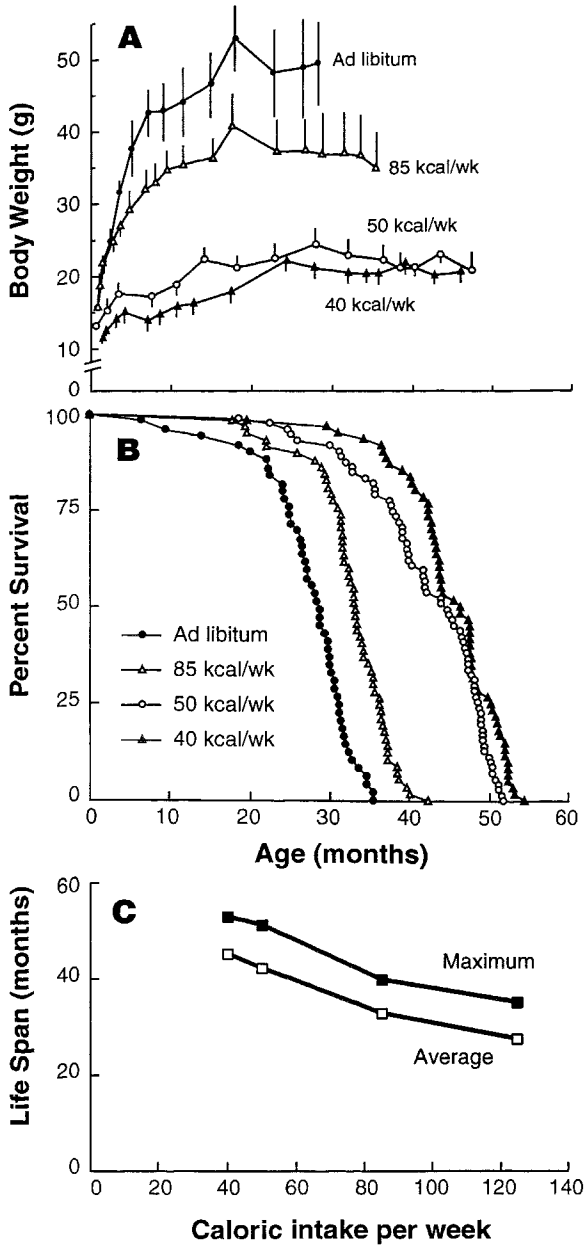


FIG. 1.—Effect of caloric intake on body weight (A), percent survival (B), and life span (C) in female C3B10F1 mice. Symbols in A also apply to B. Adapted from Weindruch et al (25).

overtly (24). An exception to this generalization is the (Fischer-344 × Brown Norway)$F_1$ rats developed at the National Center for Toxicological Research, which are both quite long-lived and leaner than either of the parent strains (R. J. Feuers, personal communication).

There are significant benefits associated with controlling the caloric intake of animals used in biomedical research (Table I). The level of CR need not be severe in order to obtain these desirable outcomes. It is probable that the use of CR increases the relevance of animal studies for human health risk assessment. Indeed, most surveys find that about one-third of the U.S. population is

TABLE I.—Advantages associated with feeding control animals slightly less than the average *ad libitum* intake.

- Allows the investigator to match caloric intakes among animals. This is significant because caloric intake is a powerful modulator of aging rate and disease susceptibility.
- Provides the opportunity to match intakes of all other dietary substances.
- Reduces the complications from obesity.
- Increases the likelihood of studying healthy old controls.

obese, whereas nearly all *ad libitum*–fed Sprague-Dawley rats are obese. Likewise, obesity can be widespread in many other of the commonly studied rodent models. Therefore, rodents on CR may provide better models to represent the 65% or so of Americans who are not obese. Also, of enormous importance to risk assessment, is that CR provides a simple way to reduce bioassay variance by controlling caloric intake (which modulates the appearance and progression of spontaneous pathology).

### *CR Initiated in Midadulthood*

It is likely that adult-onset CR is most germane from the standpoint of potential human application. The few early studies of adult-initiated CR in rodents had shown that average life span could be extended, but the data on maximum life spans were equivocal, because some studies showed a reduction, rather than extension, of maximum life span [for review, see Weindruch and Walford (23)]. We initiated adult-onset CR studies on mice utilizing conditions aimed at avoiding problems that may have confounded previous studies: (a) the CR was imposed gradually rather than abruptly, (b) the CR diet was enriched in content of protein, vitamin, and minerals to avoid potential malnourishment, and (c) animals were not obese at the onset of the experimental regimen.

Male mice of 2 long-lived strains, (C57BL/10Sn × C3H/HeDiSn)$F_1$ (referred to as B10C3$F_1$) and C57BL/6J (referred to as B6) at 12 mo of age were subjected to gradual CR or to the control diet and maintained until death (23). Overall, the B10C3$F_1$ mice on CR had a significantly increased mean life span (Fig. 2B, 36.9 vs 33.0 mo, $P < 0.001$) and maximum life span as indicated by the survival for the cohort's longest lived 10% (45.1 vs 40.6 mo, $P < 0.001$). A similar result was observed for the B6 mice (a shorter lived strain), where the mean life span was increased by 20% (Fig. 2D, 29.9 vs 24.9 mo, $P < 0.02$) and the longest lived 10% increased by 21% (38.2 vs 31.5 mo, $P < 0.02$). Also, a main cause of death in both mouse strains was lymphoma, which was delayed in onset and reduced in incidence by CR. This study provided the initial demonstration that CR started in midadulthood can extend maximum life span.

Our research group continues to explore adult-onset CR. We have attempted to identify mechanisms by which CR started at 12 mo of age can oppose the development of lymphoma in B6 mice. Two possible important actions of CR in this regard might be the reduction in circulating levels of interleukin 6 (which can stimulate B lymphocytes to proliferate) and reduced transcription of the *c-myc* oncogene in splenocytes (17). More recently, we have studied rats subjected to CR in late middle age (17

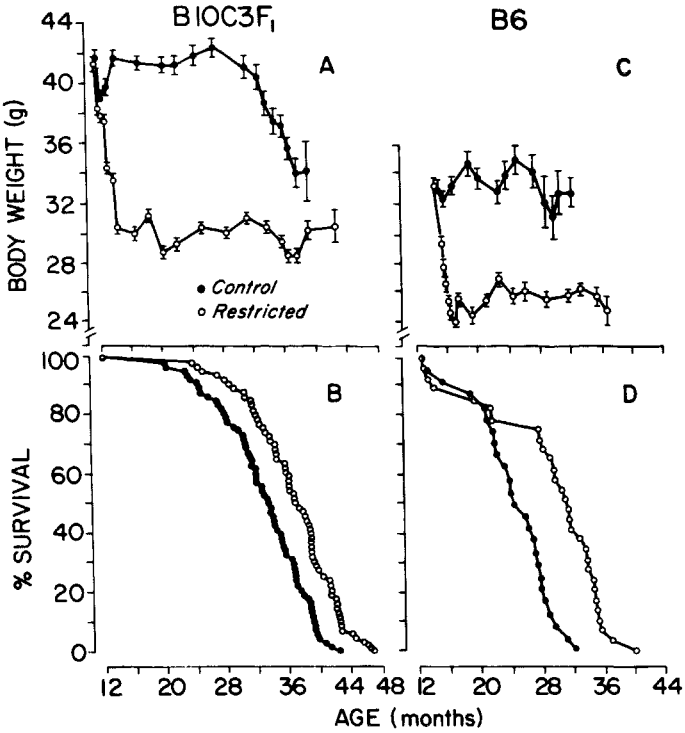


FIG. 2.—Body weights and survival of male B10C3F$_1$ mice (A and B) and B6 mice (C and D) fed control or adult-onset restricted diets (23). The food restriction started at 12 mo of age. Weights are plotted as means ± standard error for all mice alive at the indicated ages. Each point in the survival curves represents the age at death of 1 mouse.

mo of age) for selected measures in several tissues including bone (10), large intestine (13), skeletal muscle, and prostate.

*Mechanisms*

Only brief comments to this important topic are given, in part because Masoro's article in this volume focuses on CR's mechanisms in rodents and also because of the availability of a very recent review (12). As already mentioned, the clear inverse relationship between calorie intake and life span points toward the importance of mechanisms for CR having strong ties to energy metabolism. Accordingly, the mitochondrion is an organelle of long-term personal interest in the context of CR (20, 22, 24). As recently discussed (12), under normal physiologic conditions, the use of oxygen by cells of aerobic organisms generates potentially deleterious reactive oxygen metabolites. Mitochondria appear to provide a major source of reactive oxygen species as a result of the normal functioning of the electron transport system. It appears that a chronic state of oxidative stress exists in cells, especially those types such as nervous tissue, cardiac myocytes, and skeletal muscle, which share a high dependence on oxidative energy metabolism and have relatively poor repair capacities. Furthermore, the level of oxidative stress increases with age, and this increase is attenuated by CR in rodents.

## CR IN PRIMATES

Due to the obvious public health implications, the question arises whether or not CR similarly retards age-related deleterious changes and extends longevity in humans. Although presently no definitive information exists in humans, 3 studies on nonhuman primates (primarily rhesus monkeys) are currently in progress (2, 4, 7). Also, there are data from human epidemiologic studies as well as from recent experiments involving people subjected to CR that allow more informed speculation on the possibility that low energy intakes may benefit people.

*Studies in Nonhuman Primates*

These studies are evaluating the effects of CR in monkeys on a variety of biologic measures, most of which are either indicators of health status or are age sensitive. Additional measures of interest are those that are sensitive to CR in rodents. The measures include body composition, glucoregulation, metabolic rate, and immune and ocular functions. The available results suggest that a reduction of caloric intake of ~30% can be safely carried out in rhesus monkeys. Furthermore, certain physiological effects of CR that occur in rodents, including decreased blood glucose and insulin levels and improved insulin sensitivity (7), as well as lowering of body temperature (8), also occur in the restricted monkeys. Whether life-span extension is achieved in CR monkeys should become known in the future.

*Studies in Humans*

Unfortunately, because nearly all of the world's calorically restricted people are malnourished, only very limited data are available concerning humans subjected to long-term undernutrition without malnutrition. Well-controlled studies of CR's influence on the rate of aging in humans have not been conducted. Nonetheless, some indirect evidence supports the view that CR would have human efficacy. For example, the diet of many individuals on Okinawa approximates that of rodents on CR in that calorie intake is low and nutrition is otherwise adequate. The incidence of centenarians there is high, 2–40 times that of any other Japanese island (5). Additional support comes from population studies in the United States and elsewhere showing that certain cancers occur less frequently in individuals reporting low-calorie intakes (21). Also, 8 people living in a controlled, confined, and controversial environment (the Biosphere) were forced to reduce their calorie intake for ~2 yr due to lower than expected food production. However, they were able to avoid malnutrition and showed several changes in their physiology and blood chemistry like those seen in rodents subjected to CR such as decreases in weight, mean systolic/diastolic blood pressure, total serum cholesterol, serum triglyceride, fasting glucose, and leukocyte count (18).

Researchers in The Netherlands recently investigated the feasibility of implementing a moderate CR in normal-weight persons (15). The study group consisted of 24 young adult men. Sixteen of these men received 80% of their normal caloric intakes; the remaining men served as a control group and ate their normal caloric intake.

The study lasted for 10 wk. The results of the study showed significant weight loss in the restricted group and beneficial effects of the restriction on blood pressure and lipid profiles. Adverse effects were not reported. These subjects were also studied for body composition (16) and fibrinolytic factors (14) with "beneficial" alterations in the latter reported for the men on CR.

To conclude, the CR paradigm is no longer a curiosity largely investigated by a few gerontologists. Instead, the findings from CR studies appear to be of increasing importance to many disciplines. For the gerontologist, CR continues to provide a tool to learn about the basic mechanisms of aging. For the toxicologist and all others concerned with the long-term maintenance of experimental animals, CR offers a simple, yet powerful, way to control obesity and the unwanted appearance of premature disease. Arguably, caloric intake is as important to control among test animals as are other variables such as room temperature/humidity, light cycles, and pathogen status, the rigorous control of which is standard operating procedures in most animal facilities.

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