Review Article

Caloric Restriction and Aging: Controversial Issues

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It has long been held that food restriction extends the life span of rodents and other species by decreasing caloric intake and slowing the rate of aging. Recent findings challenge these concepts. This review assesses these controversial issues. The conclusion is that caloric restriction underlies the life extension of rats, but not of Drosophila. Mortality characteristics show that food restriction slows the rate of aging of rats and, in some studies, of mice. However, in other mouse studies and in Drosophila, mortality characteristics have been interpreted as indicating that it delays the start but does not slow the rate of aging; the author believes that this interpretation is faulty. These differences in mortality responses to food restriction provide a potentially powerful tool for uncovering basic mechanisms underlying its life-prolonging action. A hypothesis is presented for use in the search for these mechanisms.

Recently, based on new findings and the reevaluation of older ones, long-held concepts regarding the life-extending and related effects of caloric restriction (CR) have been challenged. The most fundamental of these challenges are examined in this review.

Does Food Restriction Extend Life by Reducing Energy Intake?

In 1935, McCay and colleagues (1) published their seminal paper showing that restricting food intake well below that of ad libitum-fed rats results in a marked increase in longevity. In a subsequent study published in 1939, McCay and colleagues (2) reported that reduction in caloric intake without reducing protein, vitamins, and minerals also extended the life of rats. Based on these findings, McCay and his associates concluded that the decreased intake of calories is the dietary factor that underlies the life-extending action of food restriction (FR). Some 50 years later, semi-synthetic diets were used in our laboratory to either restrict a specific class of nutrient but not caloric intake, or to restrict caloric intake but not a specific class of nutrient. Our findings showed that the increase in life span of rats in response to FR does not result from a reduction in the intake of protein (3,4), fat (5), minerals (5), or vitamins (6). Because of these findings, it has long been generally accepted that a decreased intake of energy is the major, if not the sole, dietary factor responsible for the life-extending action of long-term FR. In fact, in recent years, the term “calorie restriction” has to a great extent replaced the operational term “food restriction” and the vague term “dietary restriction” (DR). Although the evidence is very strong that CR underlies FR-induced life extension of rats, some caution is in order because studies have not yet been done on dietary carbohydrate restriction in the absence of CR. Also, it is not known if a reduced caloric intake underlies FR-induced life extension of mice because in that species the relevant studies have yet to be done. Very recently, Mair and colleagues (7) have presented evidence that the extension of life of Drosophila melanogaster by FR is not due to a reduction in caloric intake but rather results from a decrease in the intake of protein, fat, or both.

Articles published by Orentreich and his colleagues starting in 1993 (8–10) have shown that reducing the intake of methionine by about 80% extends life of rats. The magnitude of this life-extending action varies among the studies but is in the range of that observed in studies in which the food intake of rats is reduced by 40%. It does not appear that this increase in longevity is due to a decrease in food intake, but the work to date does not unequivocally establish this. Very recently Miller and colleagues (11) reported that markedly restricting methionine intake increases the length of life of the longest-lived mice; again the evidence excluding a role of reduced food intake is not unequivocal.

Recently, methionine restriction has received wide acclaim including speculation that it may play a role in FR-induced life extension. In my opinion, work on rats done in our laboratory makes it unlikely that this speculation is correct, at least for that species (4). We found that a long-term 40% reduction in food intake without a reduction in methionine intake extends the life of rats to the same extent as a 40% reduction of both food and methionine intake does (Table 1). Thus, it is clear that methionine intake need not be decreased for FR to markedly extend the life of rats.

Does this mean that different mechanisms underlie the life-extending action of CR and methionine restriction? The answer is not necessarily. A 40% reduction in caloric intake is a low-intensity stressor (12), and an imbalance of essential amino acids is also likely to be a low- to severe-intensity stressor depending on the magnitude of the imbalance. It is
well known that low levels of what are damaging agents at higher levels can have beneficial effects, a phenomenon known as hormesis (13). Masoro (14) hypothesized that hormesis underlies the life-extending action of CR; i.e., a moderate reduction in caloric intake increases longevity whereas marked reduction is a severe stressor resulting in a decreased longevity. Subsequently, Rattan (15,16) proposed that hormesis within the context of aging is characterized by the beneficial effects resulting from the cellular responses to mild repeated stressors, and he further explained that the underlying mechanism involves the stimulation of maintenance and repair pathways by the repeated episodes of mild stress. These views of Rattan are the basis of the concept presented in this review.

The extreme restriction of methionine in the studies of the Orentreich and Miller groups is likely to be at least a low-intensity stressor, because such an imbalance of essential amino acids undoubtedly markedly distorts protein metabolism as well as metabolism in general. Thus, hormesis may well underlie the life-extending action of this markedly reduced methionine intake; the recent article by Miller and colleagues (11) lends credence to this possibility. In further support of this view, it should be noted that a marked restriction in the intake of tryptophan, another essential amino acid, also extends the life of rats (17); Again, restricting only one of the essential amino acids results in an imbalanced intake of essential amino acids and a distorted protein metabolism. Moreover, evidence has also been accumulating in support of the concept that hormesis plays a major role in CR-induced life extension. For example, Anderson and colleagues (18) recently showed that three different low-intensity stressors (CR, heat stress, and osmotic stress) increase the replicative life of yeast and that the same pathway is involved in the case of each. Specifically, each of these stressors increases the level of pnc1, a nicotinamidase, thereby promoting the deacetylase activity of the sir2p, which, in turn, extends the replicative life of Saccharomyces cerevisiae. Although the hormetic pathway involved in the action of CR has not been delineated in mammals, inklings of such pathways have emerged such as the daily elevation of the peak concentration of plasma corticosterone in rats (12) and the enhanced expression of heat shock proteins in response to stressors (19). Thus, hormesis may well be a physiologic mechanism involved in life extension induced by both FR and the imbalanced intake of essential amino acids.

In conclusion, the evidence is strong that a reduction in caloric intake is the major, if not the sole, dietary factor responsible for the extension of life by FR in the rat. There is not sufficient information to know if this is also the case for the mouse, and there is limited information indicating that a dietary factor (or factors) other than reduction of caloric intake underlies FR-induced life extension in Drosophila melanogaster. It is also clear that an extreme reduction of methionine intake can extend the life of rats to a similar extent as can CR. Indeed, hormesis may be involved in both the life extension of rats due to CR and that due to extreme reduction in methionine intake. However, it should be noted that in addition to these two dietary manipulations, which markedly extend life, other dietary manipulations have also been found to extend the life of rodents, but less markedly. For example, 40% restriction in protein intake results in a small increase in the longevity of male F344 rats (3). In this case, the mechanism underlying the life extension probably differs from those involved in CR or the extreme reduction in methionine intake. Male F344 rats are prone to a progressive nephropathy, which is a major cause of their death, and the 40% reduction in protein intake slows the age-associated progression of nephropathy in ad libitum-fed male rats of this strain (20).

### Table 1. Life Extension of Male F344 Rats by Food Restriction (FR)

<table>
<thead>
<tr>
<th>Dietary Group</th>
<th>Median Survival Days</th>
<th>10th Percentile Survivors Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad libitum fed</td>
<td>730 (708–764)*</td>
<td>857 (819–961)</td>
</tr>
<tr>
<td>40% FR</td>
<td>936 (883–984)</td>
<td>1121 (1080–1168)</td>
</tr>
<tr>
<td>40% FR without protein or methionine restriction</td>
<td>956 (906–1028)</td>
<td>1158 (1125–1230)</td>
</tr>
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**Notes:** Data are from Masoro and colleagues (4).

*Numbers in parentheses are 95% confidence intervals; both restricted groups differed significantly from the ad libitum-fed group (*p < .001*) but not from each other at the *p < .05* level.

**Does FR Slow the Rate of Aging?**

Before addressing this question, it is probably prudent to provide a definition of the term “aging” as used in this review. It is used as a synonym for senescence; i.e., aging (senescence) is defined as the deteriorative changes, during the adult period of life, which underlie an increasing vulnerability to challenges, thereby decreasing the ability of the organism to survive. It has long been believed that FR slows the rate of aging. Initially this view was based on the fact that FR increases the maximum life span of populations (21). However, in recent years the reliability of using population maximum life span as an index of the rate of aging has been challenged. The validity of the number itself has been questioned because the larger the size of the population studied, the greater the maximum life span tends to be (22). Its use has been challenged on conceptual grounds as well (23,24). FR maintains many physiological processes in a youthful state at advanced ages (25), and this has been cited as evidence that it slows the rate of aging. However, the strength of this evidence is undermined by the lack of generally agreed upon physiological biomarkers of aging, despite sizable efforts to validate a set of such markers. The fact that FR delays the occurrence and/or slows the progression of age-associated diseases (25) has also been viewed as evidence that it slows the rate of aging. This evidence, too, is open to question because of a lack of agreement on whether age-associated diseases are an integral part of aging (26,27).

The rate of the exponential increase in mortality during adult life has long been widely accepted as a reliable measure of the rate of population aging (28); indeed, it is probably fair to say that it has long been viewed as the gold standard. However, it should be noted that in recent years the validity of its use has been challenged (29). What is frequently reported is the mortality rate doubling time (MRDT); i.e., the time it takes for the mortality rate to double
during the adult life of a population); this inversely relates to the exponential increase in mortality rate. In 1977, Sacher (30) assessed the data from several rat studies on the effect of CR on the MRDT, and found that CR markedly increased it. Based on this finding, Sacher concluded that CR extends life by slowing the rate of aging. In 1986, Holehan and Merry (31) analyzed data from four additional rat studies, and they reported that the MRDT of the ad libitum-fed rats was about 100 days, and that of the CR rats was about 200 days. In 2000, Pletcher and colleagues (32), utilizing Gompertzian and related analyses, published an in-depth assessment of the male F344 rats studied in our laboratory, which were either fed ad libitum or maintained on a CR regimen (6); it was concluded that CR extended the life of the rats primarily by slowing the rate of aging. Thus, based on these many findings from rat studies that have involved both genders of a spectrum of rat strains, it has been generally accepted that CR slows the rate of aging.

Until recently, little has been published on in-depth evaluations of the effect of FR on the mortality characteristics of species other than rats. In retrospect, the survival curves of a study using the B10C3F1 mouse by Weindruch and colleagues (33) published in 1986 suggest that FR did not slow the age-associated increase in the mortality rate of the mice after it was under way, but rather delayed its occurrence to an older age. However, CurtSing and colleagues (34) have pointed out that the slope of survival curves bears a mathematical relationship to the slope of the corresponding mortality curve, but not one that is easily grasped by visual inspection. Thus, my interpretation of the results of the study by Weindruch and colleagues must be viewed as iffy. In support of my view, Weindruch and Walford (35) state in their book The Retardation of Aging and Disease by Dietary Restriction that the slope of the mortality curve is decreased in some but not all of the DR studies carried out in their laboratory with the B10C3F1 strain of mouse. Moreover, Pugh and colleagues (36) assessed the effect on the age-specific mortality rate of FR started in male C57BL/6 mice at 12 months of age and found that the rate dropped significantly following the initiation of FR, but that the subsequent age-associated exponential increase in mortality was the same in the mice on an FR regimen as those not on such a regimen. In 2002, Pletcher and colleagues (37) and more recently Mair and colleagues (38) reported that in D. melanogaster, FR does not decrease the exponential increase in age-associated mortality, but rather it extends the life of this species by delaying—to a later age—the start of the exponential increase in mortality.

Thus, recent findings imply that the mechanism by which FR extends the life of rats, mice, and Drosophila may differ. Moreover, surprisingly, the mortality response of the mice appears to be more like that of the distantly related fruit fly than of the more closely related rat. However, caution is in order before drawing any general conclusions about mice, because information is limited and contradictory regarding the effect of FR on mortality rate kinetics in this species. As mentioned above, Weindruch and Walford (35) have pointed out that, in some of their studies with the B10C3F1 mouse strain, FR extended life by decreasing the slope of the mortality curve whereas in other of their studies, life was extended without decreasing the slope of the curve. Also, unlike the findings of Pugh and colleagues (36), Sohal and colleagues (39) found that FR markedly increased the MRDT of C57BL/6 mice. Clearly, genetics does not explain these differences.

These findings of differences in the effects of FR on mortality characteristics bring a major issue into focus. The classical interpretation of Gompertzian and related analyses is that FR does not slow the rate of aging in D. melanogaster or in mice in those cases where the MRDT is not increased. Indeed, in his chapter in the first edition of the Handbook of the Biology of Aging, George Sacher (30) used the treatment of rats with procaine as an example of a manipulation that extends life but does not slow the rate of aging; i.e., procaine treatment was found to decrease mortality at all ages but not to increase the MRDT. In my opinion, this interpretation is absurd. The fact that the age-associated exponential increase in mortality rate is delayed over a significant period of time is reason enough to conclude that the rate of aging has been slowed; i.e., this prolonged delay should be interpreted as a marked slowing of aging and that these animals differ only in the temporal pattern of CR’s action from those animals in which CR causes a smaller reduction in the age-associated increase in mortality rate but one that occurs over most of adult life. It is suggested that either of the following two scenarios be regarded as evidence for the slowing of the rate of aging: (1) a decreased age-associated exponential increase in mortality rate during most of adult life or (2) a reduced age-specific mortality rate throughout most of adult life.

**DOES FR EXTEND LIFE WHEN INITIATED AT ADVANCED AGES?**

An answer to this question is of both conceptual and practical significance. Moreover, the 2003 report of Mair and colleagues (38) has heightened its importance. They found that FR begun at advanced ages extends the life of D. melanogaster and, in addition, FR initiated at advanced ages decreases the age-specific mortality rate to that of flies that have been food restricted for their entire adult life. The latter is a mind-boggling finding, and it is imperative to know if it is also true of mammals.

The effect of late-life initiation of CR has been the subject of several rat studies. Lipman and colleagues (40) reported that CR does not extend the life of male F344 × BNF1 rats when initiated at 18 or 24 months of age, although it quite effectively extends the life of this rat strain when initiated at 14 weeks of age (41). Moreover, Lipman and colleagues (42) reported similar findings in their study of Long Evans rats. And Ross (43) reported that starting CR at 300 days of age decreases the length of life of Sprague-Dawley rats, a stock that exhibits a marked life extension when CR is started at a young age. However, Yu and colleagues (3) found that CR initiated at 6 months of age was almost as effective in extending the life of male F344 rats as when initiated at 6 weeks of age. Thus, in rats, CR results in significant life extension when started in young adulthood.
earlier, but sometime during middle age this response to CR is lost; exactly when this occurs probably depends on the strain and gender of the rat. These findings make it most unlikely that CR decreases the age-specific mortality rate of rats when initiated at advanced ages, and thus show that rats and D. melanogaster differ in this regard.

The findings on the effect of late-life FR in mice are conflicting. Dhabhi and colleagues (44) reported that when FR is initiated in male B6C3F1 mice at 19 months of age, there is decreased mortality and marked life extension. However, the findings of Forster and colleagues (45) disagree with this finding of Dhabhi and colleagues; they studied males of three mouse strains (C57BL/6, B6D2F1, and DBA/2) in which FR was initiated at 4 months of age. FR significantly extended both the median and maximum length of life of the C57BL/6 and B6D2F1 strains, but did not do so for the DBA/2 strain. Moreover, FR initiated at 17 or 24 months of age increased mortality during the subsequent 11 weeks in all three strains. In contrast, Pugh and colleagues (36) found that FR decreases the mortality rate of male C57BL/6 mice when initiated at 12 months of age.

Thus the answer to the question asked at the start of this section is yes and no. In part, this ambiguous answer may involve genetics because FR-induced life extension at an advanced age has not been observed in rat studies but has been seen with mice and fruit flies. In contrast, the findings with the C57BL/6 mouse indicate that genetic differences among mouse strains may not be the factor responsible for the contradictory findings in that species.

**DO THE DIFFERENT MORTALITY RESPONSES THAT UNDERLIE LIFE EXTENSION PROVIDE A RESEARCH TOOL?**

Aging (senescence) is due to the gradual damage to molecular structures that is not prevented by protective mechanisms or removed by repair mechanisms. Thus, CR or any other manipulation that extends life by slowing the rate of aging must do so by one of the following general mechanisms: decreasing the intensity of damaging agents that cause aging (such as decreasing the rate of generation of reactive oxygen molecules) and/or enhancing the mechanisms that either prevent the damage or its accumulation (such as increasing the response of heat shock protein systems and enhancing repair of DNA). Thus, it is worth considering whether the responses of these general mechanisms to FR are different in those animals that exhibit during adult life a long-term slowing of the age-associated increase in mortality compared to those in which the age-associated increase in mortality is delayed (i.e., markedly slowed for a significant portion of adult life) but not decreased after that.

In rats, both general mechanisms appear to be involved; i.e., in this species, there is evidence that the CR-induced slowing of the age-associated increase in mortality rate is associated with reduced levels of damaging agents and enhanced protective and repair processes. Several studies, using isolated mitochondria as well as other cell-free preparations from a variety of rat tissues, indicate that CR decreases the production of reactive oxygen molecules (46–50). However, as noted by Merry (51), a caveat is in order in that these in vitro findings have yet to be confirmed by an in vivo study. There is also strong evidence that CR protects rats from the damaging effects of a spectrum of damaging agents [e.g., heat stress, surgical stress, and toxic chemicals (52–57)] and that it enhances repair processes (58,59). Thus, in rats, the general mechanisms by which CR slows aging are on the way to being established, but even in this species much more needs to be done.

There is much less information on mice in regard to these general mechanisms. As is the case with rats, Sohal and colleagues (39) found that mitochondria isolated from C57BL/6 mice on an FR diet exhibit a decreased rate of generation of reactive oxygen molecules compared to mitochondria from ad libitum-fed mice. Also, FR protects mice from the action of a spectrum of damaging agents (60–62). Thus, it appears that the general mechanisms underlying the retardation of aging and the extension of life by CR in rats also occur in mice on an FR diet. However, this conclusion must be viewed as tentative because so few mouse strains have been studied.

Mair and colleagues (38) state that FR extends life of *Drosophila* by reducing short-term risk. However, as of yet, in this species there is not even the limited information available in rats and mice in regard to the effect of FR on the generation of damaging agents or on the enhancement of protective and repair mechanisms.

Although recent findings have been viewed as challenges to long-held concepts on the life-extending action of FR, they, in fact, provide a new base on which to expand our understanding of this phenomenon. As discussed above, two general mechanisms have been proposed as the basis of FR’s action on aging processes. One is a reduced rate of production of damaging agents, such as reactive oxygen molecules, and the second is the augmentation of protective and repair processes. The second mechanism has been linked to the ability of FR, as a low-intensity stressor, to give rise to hormesis. Indeed, as discussed above, probably hormesis is solely responsible for the life extension in response to a marked restriction of an essential amino acid. However, in the case of FR, there is evidence that both general mechanisms (i.e., a decreased level of damaging agents and hormesis) are involved. Moreover, it is likely that the relative extent of involvement of each varies among species and among animals within species. The following hypothesis is proposed as a starting point for the use of this new information as a tool for the further exploration of the influence of FR on aging and longevity: In animals in which FR induces the mortality response observed in rats, both the first and second mechanisms are significantly involved, whereas in animals in which the response has the characteristics observed in *D. melanogaster*, the second mechanism (i.e., hormesis) is primarily involved. It is further proposed that with increasing age, there is a loss of the hormesis response to FR in many but not all ad libitum-fed animals, and that when this loss is of sufficient magnitude, the initiation of CR no longer extends life.

If, as suggested in this review, hormesis is the basis of the methionine-deficient diet-induced life extension, the above hypothesis predicts that animals on this diet will exhibit
a mortality response similar to *D. melanogaster*’s response to FR. Such a study could serve as a start in the testing of this hypothesis.

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REFERENCES
Editor Nominations

The Gerontologist

The Gerontological Society of America’s Publications Committee is seeking nominations for the position of Editor-in-Chief of The Gerontologist, the Society’s multidisciplinary journal.

The position will become effective January 1, 2007. The Editor-in-Chief makes appointments to the journal’s editorial board and develops policies in accordance with the scope statement prepared by the Publications Committee and approved by Council (see the journal’s General Information and Instructions to Authors page). The Editor-in-Chief works with reviewers and has the final responsibility for the acceptance of articles for his or her journal. The editorship is a voluntary position. Candidates must be dedicated to developing a premier scientific journal.

Nominations and applications may be made by self or others, but must be accompanied by the candidate’s curriculum vitae and a statement of willingness to accept the position. All nominations and applications must be received by March 31, 2006. Nominations and applications should be sent to the Publications Committee, Attn: Patricia Walker, The Gerontological Society of America, 1030 15th Street, NW, Suite 250, Washington, DC 20005-1503.