Subfield History: Caloric Restriction, Slowing Aging, and Extending Life

Edward J. Masoro

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Caloric restriction has resulted in a consistent robust increase in the maximal length of life in mammalian species. This article reviews significant advances over the long history of research on calorie restriction and longevity.

Introduction

In an article entitled "The History of Gerontology," Jim Birren (*1*) points out that extending life and slowing or even reversing aging have been human preoccupations throughout recorded history, and he speculates that such was also the case in preliterate times. Alexander the Great spoke of a fountain of life with such properties, and Ponce de Leon purportedly discovered Florida while searching for a fountain of water said by Indian lore to have the power to rejuvenate.

At first glance, the marked increase in life expectancy in the developed nations during the 20th century suggests that this human dream has been realized. Environmental factors, such as advances in nutrition, medicine, and public health, and an increased standard of living, are believed to underlie this astonishing increase in life expectancy. However, how much, if any, of the increase in life expectancy is due to a slowing of the aging processes remains to be determined.

Gerontologists have used an increase in the maximum length of life of a population (or preferably, maximal length of life, defined as the mean age at death of the 10th-percentile survivors) as an indicator of the slowing of aging. Lowering the environmental temperature of several poikilothermic species and restricting the caloric intake of several poikilothermic and homeothermic species have been found to increase the maximal length of life of these species (*2*). There are claims that other environmental manipulations and the administration of a variety of chemical agents increase the maximal length of life, but none of these exhibit a consistently observed robust effect. Indeed, only caloric restriction has resulted in a consistent robust increase in the maximal length of life in mammalian species, specifically rats and mice.

Before 1930

In 1914, Francis Peyton Rous (who was to receive a Nobel Prize in 1966 for his work on cancer) published a paper in the *Journal of Experimental Medicine* that showed that reducing food intake inhibited the occurrence of spontaneous tumors in rodents (*3*). Although this paper did not directly address the effects of caloric restriction on longevity and aging, it was the first in a long line of reports showing that decreasing food intake retards carcinogenesis. Moreover, because the onset of most cancers is age-associated, many of those reports have addressed the issue of dietary restriction and carcinogenesis within the context of gerontology.

In 1915 and 1917, the distinguished Yale nutritionists Osborne and Mendel published findings in the *Journal of Biological Chemistry* and *Science*, respectively, on the effect of reduced food intake on aging and longevity in rats (*4*, *5*). They blunted the growth of one group of female rats by decreasing their food intake over the period from 45 days of age to 6 months of age, and they compared this group with a group whose food intake was unrestricted. The rats on restricted food intake were fertile when bred at 16 to 23 months of age, whereas those not restricted were sterile at those ages. In addition, the rats that had been restricted lived longer. These findings led to the conclusion that retarding growth delays age-associated changes in the functioning of the reproductive system and promotes longevity. These papers had less impact than one might have expected, probably for the following reasons. First, the longevity component of the study was flawed because of the premature death of some animals from disease. Second, Robertson and Ray reported in a 1920 paper in the *Journal of Biological Chemistry* that the rate of growth of mice positively correlated with the length of life (*6*).

In a 1929 issue of the *Journal of Nutrition*, McCay and associates (*7*) showed that feeding a low-protein diet slowed the growth rate of brook trout and increased their longevity. This group of Cornell nutritionists was well aware of the discrepancy in the literature in regard to the relationship between growth rate and longevity in rodents, and they decided to explore the issue further, a decision that resulted in their classic research on caloric restriction.

1930s Studies of McCay and Colleagues

In their first study, begun in 1930, they used three groups of weanling rats (*8*). One group, fed ad libitum, grew to maturity at what the investigators felt to be a normal rate. Food intake was restricted for the other two groups, so that no growth occurred until death seemed imminent, whereupon the allotment of food was increased just enough to keep the rats alive. Thus, the rats on the restricted food intake underwent long periods of no growth interspersed with periods of growth. One of the two groups was kept on the restricted diet for 700 days and the other for 900 days. The rats that grew normally had a mean length of life of about 600 days, but many of the rats in the two restricted groups lived much longer than that.

The first study of McCay and associates had involved the restriction of all components of the diet. In a subsequent study (*9*), the intake of fat and carbohydrate was restricted but not that of protein, minerals, and vitamins. Again, the group of rats on the restricted rations lived much longer.

McCay and colleagues concluded that the longer length of life of rats on the restricted diets was due to the decreased rate of growth. However, it should be noted that such a conclusion

The author is an emeritus professor in the Department of Physiology at the University of Texas Health Science Center, San Antonio, TX 78229-3900, USA. E-mail: masoro@aol.com

was based more on the belief that led the investigators to do this research rather than the actual findings of their studies.

The 1940s, 1950s, and 1960s

During these three decades, much of the research focused on the ability of caloric restriction to delay the occurrence and retard the progression of cancer. Studies in the 1940s and 1950s by research groups headed by Morris, Rusch, Saxton, Silberberg, Tannenbaum, Vischer, and White confirmed and expanded the early work of Rous, clearly establishing caloric restriction as a robust inhibitor of carcinogenesis in both mice and rats. Saxton and Kimball (*10*) reported that caloric restriction in rats also markedly slowed the progression of kidney disease, which culminates in death caused by renal failure, a major age-associated disease and cause of death in this species.

There was also some focus on aging itself. Rudzinska (*11*) reported that restricting food enhanced the longevity of a protozoan (*Tokophrya infusionum*), and Fanestil and Barrows (*12*) found that it did so in the rotifer. These studies were forerunners of the current focus on the use of invertebrate species to investigate the mechanisms underlying the anti-aging action of caloric restriction. Moreover, Alex Comfort reported that retarding the growth of fish by restricting food increased their longevity (*13*). Thus, evidence was mounting that suggested that the life-extending action of reduced food intake might be a widely occurring biological phenomenon. Also, in the 1940s and 1950s, Maurice Visscher and his colleagues at the University of Minnesota extended the work of Osborne and Mendel on the retardation of female reproductive aging by caloric restriction.

In 1960, B. N. Berg, in collaboration with H. S. Simms, reported a detailed study on the effects of long-term food restriction on fertility, longevity, and pathology in rats (*14*). Upon finding that food restriction retarded reproductive aging, increased longevity, and attenuated age-associated diseases, they hypothesized that these beneficial actions resulted from the reduction in body fat content. Although they had not measured body fat content, they surmised that animals on caloric restriction are leaner, certainly a reasonable conjecture. The apparent basis for their hypothesis was the growing evidence that obesity in humans is a health risk. Although the retardation of growth hypothesis, proposed by McCay and colleagues some 25 years earlier, remained the dominant view, the reduced body fat content hypothesis gained many adherents, particularly among nutritionists.

Beginning in the late 1950s and extending through the 1960s into the 1970s, Morris Ross and his collaborators at the Fox Chase Cancer Institute carried out a series of longevity studies on food restriction in Sprague-Dawley rats, focusing on both a detailed analysis of pathologic lesions and the manipulation of individual dietary components. A paper published by Ross in 1961, "Length of Life and Nutrition in the Rat," provides the essence of this group's work (*15*). They found that from age 6 months through 24 months, the age-specific death rate is lower in food-restricted rats than in those fed ad libitum. The McCay group's claim—that reduced caloric intake is the nutritional factor responsible for life extension—was based on weak evidence; the Ross group provided much stronger support for this view. Indeed, most of the Ross group's findings confirmed those already found by others, but the quality of their work was such that it led many to recognize the importance of caloric restriction in research on biological aging. For example, on a personal note, I heard Morris Ross speak at a National Institutes of Health (NIH)-sponsored workshop in Seattle in 1973. Although he was far from an inspiring speaker, the findings he reported, which summarized the work of his group, were so compelling that I was led to spend most of my remaining research career on caloric restriction and aging (see "Hungry for Science"*).

The Walford Group

Beginning in the 1970s, research on caloric restriction and aging markedly intensified, and Roy Walford and his colleagues at the University of California, Los Angeles, were one of the first research groups highly focused on this subject area. Because Walford had written a book entitled *The Immunologic Theory of Aging*, it is not surprising that his group's 1975 paper was on the effect of caloric restriction on immune function (*16*). They found that long-term caloric restriction enhanced the proliferative response to mitogens of splenic lymphocytes isolated from mid-life and late-life mice. This group then pioneered in many avenues of study on caloric restriction, including its effect on DNA repair, mitochondrial function, metabolic characteristics, and body temperature. Of special importance was their finding that when caloric restriction is initiated in mice at 12 months of age (early middle age), it significantly increases the life-span, although less markedly than when initiated at or near weaning (*17*) (see Weindruch Classic Paper†). Several of this group's investigators (Effros, Koizumi, Spindler, and Weindruch) subsequently went on to prestigious independent research careers in gerontology, including but not limited to the study of caloric restriction. In 1988, Weindruch and Walford coauthored *The Retardation of Aging and Disease by Dietary Restriction*‡; this book provides excellent encyclopedic coverage of research findings and played an important role in attracting talented investigators to the field. It is interesting to note that Walford was a member of the Biosphere 2 crew. Attempting to grow all their own food, they experienced unintended caloric restriction for most of the time spent in Biosphere 2. During that 2-year period, biochemical and physiological measurements were made of the crew, and these were published by Walford *et al.* in 1992 (*18*). It is striking that many of the findings in these human subjects are similar to those seen in mice and rats on caloric restriction. An advocate of the use of caloric restriction to retard human aging, Walford has published a great deal of material on this subject for lay people. This activity has drawn criticism from many, including myself, because there is no solid evidence that caloric restriction has anti-aging and life-prolonging actions in humans. Also, it seems to me that lay people with little knowledge of nutrition could well adopt food restriction programs that are harmful.

The Masoro Group

In the 1970s, our group at the University of Texas Health Science Center at San Antonio started an intensive research program on the effects of caloric restriction on the aging of specific pathogen-free (SPF) male F344 rats. Physiology and pathology were the major focuses of our studies. The use of semisynthetic diets enabled us to readily determine the effects of restricting individual components of the diet, which showed beyond doubt that the decrease in caloric intake is the major factor

* http://sageke.sciencemag.org/cgi/content/full/sageke;2003/1/nf1
† http://sageke.sciencemag.org/cgi/content/abstract/sageke;2001/1/cp12
‡ http://www.walford.com/books.htm

in the anti-aging action of food restriction. We also showed that starting caloric restriction in young adult rats (6 months of age) was as effective as that started at 6 weeks of age (2 weeks after weaning) in extending life-span and retarding age-associated pathology; in addition, we showed that restriction from 6 weeks to 6 months of age (the period of rapid growth) was not nearly as effective (*19*). These findings, along with those of the Walford group discussed above, clearly show that the long-held retardation-of-growth hypothesis of McCay is not valid. Although the rats on caloric restriction had a reduced fat content, analysis of our findings indicated that the decrease in body fat content is not an important factor in the anti-aging actions (*20*). In 1977, George Sacher proposed that caloric restriction retards aging by decreasing the intensity of energy metabolism (*21*). Although no experimental evidence was offered to support this view, many biological gerontologists immediately embraced it. Our group did not. In our studies, the rats on caloric restriction had an increased food intake per gram of body weight for most of the life-span, a finding clearly not in accord with Sacher's hypothesis (*22*) (see Masoro Classic Paper[§]). Indeed, McCarter and Palmer of our group directly addressed this issue and found that for most of the life-span, the metabolic rate per unit of lean body mass of rats on caloric restriction is not less than that of ad libitum-fed rats (*23*). Thus, the somewhat surprising conclusion is that caloric intake per rat, and not per unit of "metabolic mass," underlies the life-prolonging and anti-aging actions of caloric restriction. Two other findings of our group underlie current cutting-edge research on caloric restriction. One is the reduced plasma glucose levels and markedly reduced plasma insulin levels seen throughout the life-span of our rats maintained on caloric restriction (*24*). The other is the lifelong increase in the daily maximal level of plasma free corticosterone in calorie-restricted rats (*25*). Byung Pal Yu (see Yu Classic Paper[‖]) of our group became a leader in research on oxidative stress and caloric restriction, as will be discussed below. Also, Dike Kalu independently developed an outstanding research program on aging and bone.

Merry and Holehan

In the 1970s, Brian Merry and Ann Holehan, his wife, initiated a research program in the United Kingdom on caloric restriction, the first such program in Europe. Their work focused on reproductive function and longevity in Sprague-Dawley rats, and they reported that although female rats on caloric restriction exhibit a reduced fertility at young ages, they remain fertile at much older ages than do fully fed rats (*26*). Then they extended their work to include reproductive endocrinology and thyroid function. They were the first to investigate the influence of caloric restriction on male reproductive function and found that in contrast to the female, caloric restriction does not retard the age-associated decrease in male fertility (*27*). Merry and Holehan then teamed up with S. E. M. Lewis and D. F. Goldspink in a series of studies on the effects of caloric restriction on protein metabolism in Sprague-Dawley rats. This team found that caloric restriction increases whole-body protein turnover from age 1 year on (*28*), a most important finding as it suggested that caloric restriction might prevent age-associated cellular accumulation of damaged proteins.

The Good Group

Also in the 1970s, Bob Good and colleagues began studies on the influence of caloric restriction on autoimmune disease in mice. In their initial study, they used the B/W mouse strain that is susceptible to an autoimmune disease that leads to renal failure and death at an early age. Caloric restriction initiated at a young age was found to more than double the life-span of this mouse strain (*29*). Over the next 15 years, this group found that caloric restriction similarly affected a variety of mouse strains susceptible to autoimmune diseases, and they explored mechanisms underlying these protective effects. Gabriel Fernandes and Ed Yunis, who were involved in the early studies of this group, became distinguished independent investigators in the field of immunology and aging, and Fernandes has continued to work on basic mechanisms underlying caloric restriction's protection against autoimmune diseases.

The National Center for Toxicologic Research Program

In the 1980s, the National Institute on Aging (NIA) established a program at the National Center for Toxicologic Research (NCTR) for the production and study of calorically restricted mice and rats. Directed by Ron Hart and Angelo Turturro, this program utilized four mouse strains and three rat strains. Intramural studies focused on longevity and pathology as well as the areas of expertise of NCTR staff members, such as organismic physiology (Phillip Duffy), intermediary metabolism (Ritchie Feuers), and drug metabolism (Julian Leakey). The intramural program generated a wealth of data from well-executed studies; a summary of the survival characteristics of the mouse and rat strains has recently been published (*30*). However, by making these animals available to university investigators, the program has had an even more profound impact on caloric restriction research, because it engaged the interest of quality researchers in a variety of biological specialties in such studies. Indeed, the long-term impact has been a greatly expanded interest in and study of caloric restriction and aging.

Primate Studies

In the late 1980s and early 1990s, George Roth and collaborators at the NIA (Fig. 1) (*31*) and Joe Kemnitz and collaborators at the University of Wisconsin (*32*) initiated research on the effects of caloric restriction on the aging of rhesus monkeys. Also in the early 1990s, Barbara Hansen and Nodi Bodkin of the University of Maryland, Baltimore, began to interpret their obesity study on rhesus monkeys in terms of caloric restriction (*33*). Of course, the longevity component of all these studies remains to be completed. However, many of the physiological effects of caloric restriction in rodents also occur in rhesus monkeys (see "Monkey in the Middle"[¶]), and such similarity has led many to feel that caloric restriction will have an anti-aging action in this primate species, which will result in life-span extension. Only time will tell!

Very recently, Roth *et al.* (*34*) analyzed data from the long-standing Baltimore Longitudinal Study of Aging, and they reported that survival was greater in men who maintained lower body temperature, lower plasma insulin levels, and higher dehydroepiandrosterone levels than in others in the study. Significantly, caloric restriction is known to lower body temperature and plasma insulin levels in both rodents and rhesus monkeys and to increase

[§] http://sageke.sciencemag.org/cgi/content/abstract/sageke;2003/1/cp1
[‖] http://sageke.sciencemag.org/cgi/content/abstract/sageke;2002/37/cp18
[¶] http://sageke.sciencemag.org/cgi/content/abstract/sageke;2002/31/nw108

Fig. 1. A CR monkey is shown on the left, and a control monkey is shown on the right. Credit: This photo originally appeared in the following article: Roth *et al.*, *J. Anti-aging Med.* **1**, 315 (1998).

plasma dehydroepiandrosterone levels in rhesus monkeys. Unfortunately, information was not provided on the caloric intake of these men; thus, it is not known whether these findings are due to differences in caloric intake or environmental factors or genetics. In 2001 and 2002, in response to a Request for Applications, the NIA funded three 7-year studies on caloric restriction in humans, aimed at determining its effects on human physiology, metabolism, body composition, risk factors for age-associated pathology, and progression of age-related changes; the potential for adverse effects will also be evaluated.

Use of the Tools of Molecular Biology

Arlan Richardson and his colleagues at Illinois State University were the first to apply the tools of molecular biology to caloric restriction and aging (see Birchenall-Sparks Classic Paper[#]). In work published in 1987, they compared the expression (protein synthesis, mRNA levels, and nuclear transcription) of α_{2u}-globulin by hepatocytes isolated from 18-month-old rats fed ad libitum with that of rats on caloric restriction. Protein synthesis, mRNA levels, and nuclear transcription were increased two- to threefold by caloric restriction (*35*). In 1996, Richardson was named director of the Aging Research and Education Center that I had initiated at the University of Texas Health Science Center at San Antonio. In this position, he has continued to lead the way in the development of molecular biology technology, including the use of transgenic mouse models, for the study of aging in general and caloric restriction in particular (see Movies[**]). During the past 10 years, molecular biology technology has become the major tool in the study of the biology of aging, including the anti-aging action of caloric restriction (regrettably, resulting in the near exclusion of other approaches; see Martin Viewpoint[††]). Thus far, only a few genes have been carefully studied. However, this deficiency is being partially addressed by the recent development of high-density gene expression array technology, which permits the simultaneous analysis of the concentrations of the mRNA transcripts of thousands of genes. Rick Weindruch and his colleagues at the University of Wisconsin were the first to use this technique for the study of aging and caloric restriction, and they summarized their findings in a recent review article (*36*). It must be pointed out that gene-profiling technology does have a good many limitations. Only twofold differences in mRNA transcript levels can be reliably determined, and it is possible, and in my view likely, that more subtle differences may well play the major role in senescence. Also, the investigators using the method seem to have forgotten that a change in the mRNA level of a transcript does not necessarily mean a change in the expression of that gene (that is, an increase in the concentration of the corresponding protein). Moreover, mixed cell types that coexist in a particular tissue are analyzed together, which leads to a tenuous interpretation of the functional meaning of the findings. Furthermore, up to now, the number of animals used in studies employing the array technology has been very small.

To be sure, there are also serious limitations in the use of the transgenic mouse model to learn about caloric restriction and aging. First is the difficulty of knowing whether the produced genetic alteration has effects other than the expected one. Second, as it has been used, the genetic alteration produced is expressed throughout life and thus can affect development as well as aging. Fortunately, there is a technology that circumvents the second limitation, but, to my knowledge, it has not yet been used successfully in the study of caloric restriction and aging. Thus, further development of molecular biology technology is needed before it can achieve the ambitious goals of its advocates.

Current Research Focus

Although other important work is ongoing, such as the research on nonhuman primate species, much of the current research focus is on biological mechanisms underlying the anti-aging action of caloric restriction. Most studies are aimed at addressing one or more of the following hypotheses:

Oxidative Damage Attenuation Hypothesis

Intrinsic living processes, such as energy metabolism, and environmental factors, such as cigarette smoking, cause the continuous generation of reactive oxygen molecules (see "The Two Faces of Oxygen"[‡‡]). These molecules can oxidatively damage the molecular structure of organisms (an action referred to as oxidative stress), and such damage has been found to accumulate with advancing age. In 1996, Raj Sohal and Rick Weindruch (*37*) and Byung Pal Yu (*38*) independently proposed that the anti-aging action of caloric restriction stems from the attenuation of the age-associated increase in oxidatively damaged molecules. Indeed, it has been amply demonstrated that caloric restriction decreases the age-associated accumulation of oxidatively damaged lipids, proteins, and nucleic acids. However, what has not been clearly demonstrated is the role of such damage in senescence. Thus, until it can be established that oxidative stress plays a major role in aging, the validity of the attenuation hypothesis remains unproven.

Alteration of the Glucose-Insulin System Hypothesis

As discussed above, caloric restriction causes a lifelong decrease in plasma glucose and insulin levels. On the basis of these findings and of the fact that long-term hyperglycemia and hyperinsulinemia have damaging effects similar to those occurring in senes-

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cence, I proposed in 1996 that the anti-aging action of caloric restriction is due to the sustained reduction of glycemia and insulinemia (*39*). The initial response of investigators to this hypothesis was to focus on glycemia, probably because Tony Cerami had proposed that increasing glycemia promotes the glycation of proteins and nucleic acids, thereby altering their function so as to cause aging (*40*). Although it has been demonstrated that caloric restriction decreases glycation and the accumulation of advanced glycation end products, there is as yet no evidence that the effect on glycation plays a major role in the anti-aging action of caloric restriction. Recently, the focus has shifted to insulinemia because of studies on nematodes (*Caenorhabditis elegans*), fruit flies (*Drosophila melanogaster*), and yeast (*Saccharomyces cerevisiae*) by Cynthia Kenyon's group (*41*) (see Sonntag Perspective[§§]), Linda Partridge's group (*42*) (see "The Road More Traveled[‖‖]"), and Leonard Guarente's group (*43*) (see "High-Octane Endurance—Yeast in the Metabolic Fast Lane Live Longer[¶¶]"), respectively (see also Hekimi Review[##]). They found that the life of these organisms was markedly extended by loss-of-function mutations in genes coding for components of the cellular insulin-like signaling pathway. These investigators and others suggested that the extension of life of mice and rats by caloric restriction involved a similar mechanism. My first reaction to this thinking was emphatically negative. How could they make such a claim when it was known that caloric restriction enhances insulin action in the skeletal muscle of these species (*44*)? Well, the work of the Ruvkun group has made me reconsider; they found that a loss-of-function mutation in an insulin signaling pathway gene caused life extension in *C. elegans* if it occurred in the nervous system but not if it occurred in muscle or intestine (*45*). Therefore, although I now feel that this hypothesis is worthy of further study, I still find its proponents to be what Alan Greenspan might call "irrationally exuberant."

Alteration of the Growth Hormone-IGF-1 Axis Hypothesis

Caloric restriction is known to influence the growth hormone-insulin-like growth factor 1 (-IGF-1) axis. It decreases growth hormone secretion and plasma growth hormone concentrations in rats at young ages but not at advanced ages (*46*), and it also lowers plasma IGF-1 concentrations at young ages but less so with advancing age (*47*). In a 1999 paper, Sonntag *et al.* (*48*) suggested that these alterations in the growth hormone-IGF-1 axis might underlie much of the life-prolonging and anti-aging actions of caloric restriction in the rat and mouse models. Recent work on dwarf mice (see Bartke Viewpoint[***]) and genetically engineered mice provides some support for this hypothesis (see Ames mice[†††], Snell mice[‡‡‡], Laron mice[§§§], and Little mice[‖‖‖]). Dwarf mice with pituitary glands devoid of growth hormone-producing cells exhibit a markedly extended life-span (*49*) as do genetically engineered mice with a targeted disruption of the growth hormone receptor, which results in low concentrations of plasma IGF-1 (*50*). Also, because fruit flies and nematodes do not have separate insulin and IGF-1 signaling pathways, the genetic studies with these species, discussed above in support of the "alteration of glucose-insulin system hypothesis," similarly support the "alteration of the growth hormone-IGF-1 axis hypothesis." However, Bartke *et al.* (*51*) have concluded that the mechanism underlying the long life-span of these dwarf mice differs from that responsible for life extension due to caloric restriction (see "Dieting Dwarves Live It Up[¶¶¶]"). This conclusion was based on two findings. First, restricting the caloric intake of these dwarf mice causes a further increase in life-span, but Clancy *et al.* (*52*) have pointed out that this fact does not necessarily mean that different mechanisms are involved (see "The Road More Traveled[###]"). Second, the shape of the survival curve of a population of these dwarf mice indicates that senescence is delayed, whereas the shape of the survival curves of rodents on caloric restriction indicates a slowing of the rate of senescence. Although the evidence in support of the alteration of the growth hormone-IGF-1 axis hypothesis is not conclusive, further exploration of this hypothesis is clearly warranted (see "One For All[****]").

Hormesis Hypothesis

In 1998, I proposed that hormesis[††††] underlies the anti-aging and life-prolonging actions of caloric restriction (*53*). Hormesis has been defined in various ways and the following is the definition I used when formulating my hypothesis: Hormesis is the beneficial action resulting from the response of an organism to a low-intensity stressor. Caloric restriction in rats causes a moderate life-span increase in the daily levels of plasma free corticosterone, which indicates that it is a long-term low-intensity stressor. The fact that it enhances the ability of rats and mice of any age to cope with a spectrum of acute intense damage, such as that due to surgery or exposure to toxic chemicals, points to a hormetic action. If aging is primarily the result of damage caused by intrinsic living processes and environmental agents, which is not repaired, then it is reasonable to propose that caloric restriction protects against this damage in a similar fashion. The question then is how does the low-intensity stressor action of caloric restriction increase protection against damaging agents? The daily elevation of plasma free corticosterone levels may well be such a mechanism. In addition, caloric restriction enhances the induction of stress proteins in response to damage, and this is also a likely mechanism (*54*). Further support for the "hormesis hypothesis" is provided by the findings that single-gene mutations that extend the life of invertebrate species also increase the ability of these organisms to cope with damaging agents (*55*).

Testing Evolutionary Hypotheses

Recently, research has also been aimed at testing hypotheses about the evolution of the anti-aging and life-prolonging actions of caloric restriction. Over the years, there had been surprisingly little work in this area. In 1989, Harrison and Archer (*56*) proposed that in nature, periods of low food availability (for example, drought) may outlast the reproductive life-span of such species as mice and rats, and that there would be enormous selective advantage for those with genomes enabling them to respond by slowing reproductive aging (and, presumably, aging in general). Phelan and Austad (*57*) challenged this "female reproductive life-span hy-

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pothesis" on the grounds that reproductive senescence would be largely irrelevant to life in the wild‡‡‡‡. In the same year, Holliday (*58*) proposed the "energy apportionment hypothesis," which is based on Kirkwood's "disposable soma theory of aging" (*59*). Holliday's hypothesis proposes that there is clearly a selective advantage for individuals with genomes that respond to food shortages by directing energy expenditure to somatic maintenance rather than reproduction. They would have an increased ability to survive the shortage and generate progeny when food again becomes plentiful. Moreover, when the reduction in food availability is sustained, as in caloric restriction laboratory protocols, this diversion of energy to somatic maintenance serves to slow senescent processes. Masoro and Austad embraced this hypothesis and extended it in two regards (*60*). First, we pointed out that well-known responses have evolved in regard to predictable food shortages, such as seasonal reproductive cessation, torpor, and hibernation; thus, the anti-aging actions of caloric restriction must have evolved in response to unpredictable food shortages. Second, because survival depends on seeking what little food is available, an increased confrontation with a host of stressors is inevitable, and therefore we proposed that an important component of this evolutionary adaptation is the diverting of energy to cope with stressors. This view is clearly in line with the hormesis hypothesis just discussed. In 1997, Walford and Spindler (*61*) proposed the "hibernation-like hypothesis" of the anti-aging action of caloric restriction. This hypothesis posits that the functional characteristics of caloric restriction and those of hibernation are two elements of a spectrum of evolutionary adaptations to food scarcity.

Although predictions can be generated from each of the evolutionary hypotheses, such predictions, surprisingly, have not been tested until recently. In 2000, Shanley and Kirkwood (*62*) reported that they did such testing by means of computer modeling of a virtual wild mouse. The results of their study were in accord with the energy apportionment hypothesis. In another study aimed at testing evolutionary theories, Kirk (*63*) reported in 2001 his findings on 10 species of rotifers. The results were also in accord with the energy apportionment hypothesis.

Future Research Directions

Looking back at the history of caloric restriction research, it seems that predicting the direction of future research is not likely to prove fruitful. New findings and new technologies have continuously changed the direction of this field of research, and there is every reason to believe that they will continue to do so. In my view, there are several immediate research priorities. One is the completion of the longevity and pathology studies of the nonhuman primates. Another is testing the four hypotheses about the biological mechanisms underlying the anti-aging actions that have just been discussed. A third is the use of new technologies to explore old hypotheses of merit. For example, imaging technology makes possible longitudinal studies of specific fat depots, thus enabling further testing of Berg and Simms's decreased body fat content hypothesis. Also, the developing technology that permits the measurement of the metabolic rates of specific organs in situ should provide definitive data regarding Sacher's metabolic rate hypothesis, which has remained popular in spite of the evidence against it. Last, but certainly not least, is further testing of hypotheses on the evolution of the anti-aging action of caloric restriction.

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