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Overview of caloric restriction and ageing

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Abstract

It has been known for some 70 years that restricting the food intake of laboratory rats extends their mean and maximum life span. In addition, such life extension has been observed over the years in many other species, including mice, hamsters, dogs, fish, invertebrate animals, and yeast. Since this life-extending action appears to be due to a restricted intake of energy, this dietary manipulation is referred to as caloric restriction (CR). CR extends life by slowing and/or delaying the ageing processes. The underlying biological mechanism responsible for the life extension is still not known, although many hypotheses have been proposed. The *Growth Retardation Hypothesis*, the first proposed, has been tested and found wanting. Although there is strong evidence against the *Reduction of Body Fat Hypothesis*, efforts have recently been made to resurrect it. While the *Reduction of Metabolic Rate Hypothesis* is not supported by experimental findings, it nevertheless still has advocates. Currently, the most popular concept is the *Oxidative Damage Attenuation Hypothesis*; the results of several studies provide support for this hypothesis, while those of other studies do not. The *Altered Glucose–Insulin System Hypothesis* and the *Alteration of the Growth Hormone–IGF-1 Axis Hypothesis* have been gaining favor, and data have emerged that link these two hypotheses as one. Thus, it may now be more appropriate to refer to them as the *Attenuation of Insulin-Like Signaling Hypothesis.* Finally, the *Hormesis Hypothesis* may provide an overarching concept that embraces several of the other hypotheses as merely specific examples of hormetic processes. For example, the *Oxidative Damage Attenuation Hypothesis* probably addresses only one of likely many damaging processes that underlie aging. It is proposed that low-intensity stressors, such as CR, activate ancient hormetic defense mechanisms in organisms ranging from yeast to mammals, defending them against a variety of adversities and, when long-term, retarding senescent processes.

Keywords: Life extension; Senescence; Oxidative damage; Hormesis; Insulin-like signaling

1. Introduction

Early in the 20th century, Osborne et al. (1917) published a paper in *Science,* which showed that decreasing the food intake of rats slowed growth and increased length of life. This paper had little impact because of the poor quality of the survival component of the study, and because Robertson and Ray (1920) reported in the *Journal of Biological Chemistry* that growth rate and length of life are positively associated in mice. However, in the 1930s, McCay et al. (1935) carried out well-executed studies clearly showing that markedly restricting food intake of rats, at or soon after weaning, resulted in life extension. Since then, restricting food intake has been observed to increase both the mean and maximum life span in a spectrum of rat and mouse strains and in many other species, including yeast, invertebrate animals, fish, hamsters, and dogs (Masoro, 2002).

McCay et al. (1939) also reported work suggesting that the dietary factor responsible for the increase in longevity is probably a decreased intake of energy. More recently, our studies, in which rats were fed semi-synthetic diets (Iwasaki et al., 1988; Masoro et al., 1989), strongly supported the conclusion that the life extension is due to a reduced caloric intake rather than reduction in a specific nutrient.

Nevertheless, this view has been challenged because of findings that show that the restriction of a specific dietary component without a decrease in caloric intake can result in life extension. However, research in our laboratory on the

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male F344 rat indicates that such findings do not necessarily invalidate the conclusion that life extension in response to a reduction in food intake is primarily due to a decrease in energy intake. For instance, in an early study (Yu et al., 1985), we found that when dietary protein is reduced by 40% with no reduction in energy intake, there is a significant life extension in ad libitum-fed rats, although not nearly so marked as that resulting from a 40% reduction in food intake. This led us to believe that part of the life extension resulting from a reduced food intake was due to the decreased intake of protein. However, in a subsequent study (Masoro et al., 1989), we found that restricting the caloric intake by 40% resulted in the same magnitude of life extension whether or not protein intake was restricted. The reason for the life extension by the 40% restriction of dietary protein in the earlier study probably relates to the fact that kidney failure in ad libitum-fed males of this rat strain is the major cause of death; in contrast, this disease process is not a significant cause of death in rats on energy-restricted diets. Since the full life-extension effect of food restriction occurs whether or not protein is restricted, it is clear that reduction in energy intake rather than in protein intake most likely plays the major role in the life extension.

The recent paper by Zimmerman et al. (2003) reporting that restriction of dietary methionine markedly extends the life of rats has also been heralded as evidence that life extension in response to a reduced food intake is due to the restriction of specific nutrients rather than calories. Those who make this claim ignore the study of Masoro et al. (1989), which clearly shows that restriction of methionine intake is not involved in the food restriction-induced life extension.

Thus, although the restriction of various nutrients can extend life, it is most likely, based on current knowledge, that life extension due to reduced food intake results from the reduced intake of calories. Indeed, the life-prolonging manipulation of restricting food intake, initially referred to as food restriction (a good operational name) and then by the vague name, dietary restriction (DR), is now usually referred to by the more specific name, caloric restriction (CR). As just discussed, however, there is not total agreement that such a specific name is warranted.

2. CR and ageing

Does the extension of life mean that CR slows the ageing process(es)? Before addressing this question, we need to define what we mean by ageing. In this paper, ageing and senescence are used as a synonyms and are 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. Thus, the question becomes: does CR slow senescence processes? That CR increases the median length of life of a population is not an evidence that it slows the ageing processes, since that would occur if only premature deaths unrelated to ageing were prevented. The fact that CR increases the maximum life span of a population has long been felt to indicate that it retards senescence. However, Gavrilov and Gavrilova (1990) have questioned, on several grounds, the reliability of using the maximum length of life as an index of the rate of ageing. A more reliable index is the average age at death of the 10th percentile survivors, which Lewis et al. (1999) call the maximal length of life; they have shown that the maximal length of life is increased by CR in both genders of the several mouse and rat strains they studied.

Compared to ad libitum-fed rodents of the same age, the physiologic processes of old rodents maintained on a CR regimen are more like those of young rodents (Masoro, 2002). This has been viewed as evidence that CR slows senescent deterioration. However, there are two concerns regarding this interpretation. The first concern is that there are two general ways in which CR brings about a more youthful physiology at advanced ages. One is by slowing the age-associated change; e.g., CR does not influence the serum cholesterol level in young adult rats, but it markedly attenuates the age-associated increase in serum cholesterol level (Liepa et al., 1980). The other is by altering the physiologic activity in the young adult, but not altering the rate of age-associated change; e.g., CR enhances hepatic proteolytic capacity in young adult rats, but it does not alter the subsequent rate of the age-associated decrease in proteolysis; thus, the livers of old rats on a CR regimen have a proteolytic capacity similar to that of young ad libitum-fed rats (Ward, 1988). The first of these two general ways of modulating a physiologic process in animals of advanced age has intuitive appeal as an indicator of the slowing of the rate of ageing. It is not obvious that such can be said for the other general way. The second concern – the overriding one – is that studies aimed to determine biomarkers of ageing have yet to establish any age-change in a physiologic process as a valid biomarker of ageing. Indeed, the lack of valid biomarkers of ageing is a serious impediment to biogerontologic study; however, the findings of Miller (2001) indicate that it may be possible for physiologic processes to provide such biomarkers.

The influence of CR on psychologic parameters is unclear. For example, several studies indicate that CR retards or delays the age-associated decline in cognition while several other studies find CR to be ineffective in this regard (Masoro, 2002).

CR delays the onset and/or slows the progression of most age-associated diseases, including neoplastic diseases, degenerative diseases, and immune diseases (Maeda et al., 1985; Bronson and Lipman, 1991; Roe et al., 1995). This action has also been viewed as evidence that CR retards the ageing processes. However, this conclusion is open to question, too, because of the ongoing debate on whether age-associated disease is an integral part of the ageing process (Hayflick, 2004; Holliday, 2004).

Probably, the most accepted method of determining if a manipulation has influenced ageing is by Gompertzian and related analyses, which provide an index of the rate of ageing by assessing the rate of the age-associated increase in age-specific mortality. Using the Gompertzian analysis, Holehan and Merry (1986) assessed the average mortality rate doubling time with data from four published studies; they found it to be 102 days for ad libitum-fed rats and 203 days for CR-rats. Pletcher et al. (2000) analyzed a study carried out in our laboratory on male F344 rats (Yu et al., 1982) and they concluded that the increase in the longevity of the rats on a CR regimen results primarily from a decreased rate of increase in age-specific mortality. Thus, the findings on rats strongly support the concept that CR extends life by slowing the rate of ageing. However, at least one study of mice does not support this view. Weindruch et al. (1986) found in their study of female C3B10RF$_1$ mice that CR delayed the start of the age-associated increase in age-specific mortality rate, but did not influence the rate once the increase was underway. This finding leads to the interpretation that in this gender and strain of mice, CR delays the start of senescence, but does not slow it once underway. Unfortunately, the number of mouse strains analyzed in this way has been insufficient to determine if this finding is merely strain-specific. However, if generally true of mice, it would suggest that the underlying mechanism by which CR influences aging differs between mice and rats. In contrast, it is striking that both among rat strains and among mouse strains, CR causes similar increases in the median and maximal length of life (Lewis et al., 1999). Further complicating the picture is the study of *Drosophila* by Mair et al. (2003), they found that within two days of switching fully fed flies of any age to a CR diet, the age-specific mortality rate decreases to that of the flies on a lifelong CR regimen.

Indeed, there is other evidence that the nature of the life-extending action of CR may differ among species. For example, Lipman et al. (1998) reported that CR does not extend the life of F344 × BNFl rats when initiated in late middle age (18 months of age) or in old age (26 months of age); they had previously reported similar findings for the Long–Evans rat strain (Lipman et al., 1995). In contrast, Dhahbi et al. (2004) found that CR increases the life span of B6C3F1 mice when initiated at 19 months of age.

3. Biological mechanism: hypotheses

Since 1935, many mechanisms have been proposed as the biological basis of the life-prolonging and ‘‘anti-ageing’’ actions of CR. Although none is strongly supported by available evidence, most continue to have advocates. Indeed, it is entirely possible that the actions of CR involve a combination of suggested mechanisms. The following is a description and evaluation of what this author considers to be or to have been the major hypotheses over the years.

3.1. Retardation of Growth Hypothesis

In the 1935 paper cited above, McCay et al. proposed that CR increases the longevity of rats by retarding growth. This hypothesis held sway until the 1980s with many gerontologists modifying it to include the retardation of development, because ageing was then viewed as a continuation of development. This hypothesis was challenged by studies published in the 1980s by Roy Walford’s group and our group. Weindruch and Walford (1982) reported that CR initiated in mice at 12 months of age significantly extends life, but not as markedly as CR initiated at or soon after weaning. In our study (Yu et al., 1985), the following four groups of male F344 rats were studied: rats fed ad libitum throughout life; rats in which CR was initiated at 6 weeks of age (2 weeks post-weaning); rats on CR from 6 weeks to 6 months of age (the rapid growth period) and fed ad libitum thereafter; rats fed ad libitum until 6 months of age (the age at which skeletal development is almost complete in this rat strain) and on CR for the rest of life. The longevity data from our study are summarized in Table 1. There are two important findings: first, when CR was limited to the rapid growth period, it did not markedly increase the age of the 10th percentile survivors; and second, when CR was initiated after the rapid growth period, it was almost as effective in increasing the age of the 10th percentile survivors as CR initiated at 6 weeks of age.

For many years, these two studies were viewed as tests that negated the *Retardation of Growth Hypothesis*. Recently, however, this hypothesis has been resurrected because of studies such as that reported by Miller et al. (2002), which shows that in a genetically heterogeneous mouse population, body weight at 2 months of age is a significant predictor of longevity; indeed, body weight from 2 to 24 months of age was shown to inversely correlate with longevity, a correlation that becomes weaker with increasing age. As can be seen from Table 2, such a correlation was not found in a study with the genetically homogeneous inbred males of the F344 rat strain (Yu et al., 1982). Thus, it seems likely that the findings of the inverse correlation in the Miller et al. (2002) study relate to genetics and are not relevant in regard to CR. However, since the nature of the life-extending action of CR may differ between mice and rats, it is possible that the *Retardation of Growth Hypothesis* is applicable to mice, but not to rats.

Table 1
Age of initiation and time period of CR and longevity of male F344 rats

Dietary group	Period on CR	Survival in days	
		Median	10th percentile
1	None	701	822
2	From 6 weeks of age	1057	1226
3	6–26 weeks of age	808	918
4	From 26 weeks of age	941	1177

Note: This table was generated from data in the report of Yu et al. (1985).

Table 2
Body weight and the length of life of ad libitum-fed male F344 rats ($n = 115$)

Age (month)	Body weight–longevity correlation	
	r	P
2	−0.14	n.s.
4	−0.15	n.s.

Note: This table was generated from data in the report of Yu et al. (1982).

3.2. Reduction of Body Fat Hypothesis

Berg and Simms (1960) hypothesized that CR's life extension is due to a decrease in body fat content. They based this view on the fact that body fat is associated with premature death in humans and on the assumption that CR decreases the body fat content of rodents, which they did not measure. It was subsequently shown that CR does decrease body fat content of rats and mice (Bertrand et al., 1980; Harrison et al., 1984; Garthwaite et al., 1986) and that it is particularly effective in decreasing visceral fat (Barzilai and Gupta, 1999). CR has similar effects on body fat of rhesus and cynomolgus monkeys (Hansen and Bodkin, 1993; Lane et al., 1997; Cefalu et al., 1997; Colman et al., 1999).

Gerontologists did not embrace this hypothesis because in the 1960s and 1970s, most accepted the *Retardation of Growth Hypothesis* as fact. However, many nutritionists viewed it favorably until two studies, published in the 1980s, provided a strong case against the validity of the *Reduction of Body Fat Hypothesis*. Bertrand et al. (1980) reported no correlation between the body fat mass and the length of life of ad libitum-fed male F344 rats and a positive correlation in male rats of this strain maintained on a CR regimen. Harrison et al. (1984) compared obese (*ob*/*ob*) mice with lean mice that were congenic except for the *ob*/*ob* locus. The length of life of the ad libitum-fed *ob*/*ob* mice was less than that of the ad libitum-fed lean mice, but *ob*/*ob* mice on a CR regimen lived longer than the ad libitum-fed lean mice, even though they had a 48% fat content compared to 22% for the lean mice. Indeed, the *ob*/*ob* mice on a CR regimen lived at least as long as CR lean mice (13% body fat).

These findings led to a dismissal of the *Reduction of Body Fat Hypothesis* until 1999 when Barzilai and Gupta (1999) revisited it in a theoretical paper, which contained no supporting empirical data. Subsequently, Blüher et al. (2003) reported that the FIRKO mouse (in which the insulin receptor is "knocked out" only in the adipose tissue) exhibits life extension and a decreased body fat mass. Although they had not studied CR, the authors came to the surprising conclusion that a reduction in fat mass, rather than a restriction of calories, underlies the food restriction-induced life extension. Recently, the Guarente group has drawn a similar conclusion based on research carried out during the past 5 years in their and David Sinclair's laboratories, which indicates that sirtuin proteins play an important roll in the life-extending action of CR. In 2000, the Guarente group (Lin et al., 2004) reported that a sirtuin protein, SIR2, is required for CR to extend the replicative life span of a yeast species (*Saccharomyces cerevisiae*) and that this action involves the deacetylase activity of this protein. Subsequently, the Guarente group and the Sinclair group reported findings indicating that it is likely that sirtuin protein deacetylase activity is involved in CR-induced life extension in invertebrate animal species as well as in mammalian species (Picard et al., 2004; Wood et al., 2004). In addition, Picard et al. (2004) found that sirtuin deacetylase activity decreases fat deposition and increases fat mobilization, thereby decreasing fat mass in mammalian white adipose tissue. Based on this finding and without studying CR, the Guarente group concluded that a major factor in CR's life-extending action is the reduction of body fat mass. Although these findings are interesting, some empirical support for their relevance in CR-induced life extension is sorely needed before the *Reduction of Body Fat Hypothesis* can be reinstated. Unfortunately, the mouse study by Miller et al. (2002), which showed an inverse correlation of body mass and longevity, did not include the measurement of body fat content.

3.3. Reduction in Metabolic Rate Hypothesis

Sacher (1977) proposed that CR extends the life span by decreasing the metabolic rate. This hypothesis was based on the many studies showing that reduction of food intake in humans decreases the metabolic rate (for a review of these studies see Garrow, 1978) and the work of Rubner (1908) showing a mammalian interspecies inverse relationship between species longevity and its metabolic rate per kilogram body mass. Pearl (1928) proposed the "rate of living theory of ageing," which extends Rubner's concept to include "longevity differences within the same species".

The findings of ongoing research in our laboratory, using male rats of the F334 strain as the animal model, were not in accord with Sacher's hypothesis. We found that following the initiation of CR, there was only a brief period of reduced food intake per gram body mass; this was followed by a lifetime of intake greater per gram body weight in the CR rats than in the ad libitum-fed rats (Masoro et al., 1982). Indeed, assuming that the lifetime intake of calories is a valid index of lifetime caloric expenditure, CR rats had a markedly greater lifetime caloric expenditure per gram body weight than did ad libitum-fed rats (Table 3), a finding clearly not in accord with the concepts of Rubner or Pearl.

Expanding our study, McCarter and Palmer (1992) determined the lifetime 24-h metabolic rate of male F344 rats under usual living conditions. A brief period of reduced metabolic rate followed the initiation of CR at 6 weeks of age. However, the metabolic rate of rats on a CR regimen quickly increased to a rate higher per gram body weight than that of those fed ad libitum, while on a per gram lean body mass, the rate was the same as those fed ad libitum. The transient decrease in metabolic rate relates to the period of

Table 3
Lifetime caloric intake

Rat group	Mean length of life days	Mean lifetime caloric intake (kcal/g body mass)
Ad libitum-fed	701	91.5
CR	986	133.5

Note: This table was generated from data in the report of Yu et al. (1982).

time in which the lean body mass falls following initiation of CR. After a relatively brief period, the decrease in lean body mass matches the decrease in caloric intake and, at this point, the lean body mass is stable. Duffy et al. (1991) reported similar findings for mice. In two long-term studies of CR in rhesus monkeys, the metabolic rate per gram of lean body mass decreased following the initiation of CR, but rose to that of non-restricted monkeys as the CR regimen continued (Lane et al., 1996; Ramsey et al., 1996). Other studies, such as the rhesus monkey study of DeLaney et al. (1999), have shown that CR results in a sustained decrease in the metabolic rate. Nevertheless, strongly weighing against the *Reduction of Metabolic Rate Hypothesis* is the fact that there are rodent studies showing that CR can extend life without a long-term reduction in metabolic rate.

In spite of these findings, this hypothesis still has many advocates. And one does, indeed, encounter the following question when dismissing it: when comparing rats on CR to those fed ad libitum, is it appropriate to normalize the metabolic rate for body size by expressing the findings per unit of either body mass or lean body mass? The confounder is that the relative sizes of tissues and organs within the rodent are not the same for animals on CR as those fed ad libitum (Yu et al., 1982). Greenberg and Boozer (2000) tried to address this confounder by expressing the metabolic rate per unit of the combined mass of heart, kidneys, brain, and liver; using this normalization method, they found that 22-month-old male F334 rats on a CR regimen and those fed ad libitum have the same metabolic rate. However, Gallagher et al. (2003) point out that the methods so far used in CR studies to normalize for body size are based on dubious assumptions. They assert that what is required are in vivo measurements of the specific metabolic rate of individual organs and tissues. Although the question of the effect of CR on metabolic rate remains yet to be answered, the recent report of Speakman et al. (2004) indicates that Sacher's *Reduction of Metabolic Rate Hypothesis* is not likely to be correct. They reported a positive correlation between metabolic rate and longevity in mice, a finding that challenges both Sacher's hypothesis and Pearl's ''rate of living theory of aging''.

3.4. Oxidative Damage Attenuation Hypothesis

Harman (1956) proposed that ageing is due to damage caused by free radicals. With the recognition that the metabolic use of oxygen is the major biological source of free radicals (e.g., hydroxyl and superoxide radicals) as well as other damaging reactive oxygen molecules (e.g., hydrogen peroxide), Harman's theory evolved into the oxidative stress theory of ageing. It also gave rise to the mitochondrial theory of ageing, since mitochondria are the major source of reactive oxygen molecules (Beckman and Ames, 1998; Barja, 2000). These reactive oxygen molecules can damage important biological molecules, including DNA, proteins and lipids, thereby altering cellular functions. Currently, many believe that the accumulation of oxidative damage is the primary basis of ageing. Although several investigators have theorized that CR retards aging and extends life by slowing the age-associated increase in oxidative damage, Sohal and Weindruch (1996) have provided an especially clear and succinct presentation of this concept, which I call the *Oxidative Damage Attenuation Hypothesis*.

Indeed, it is well established that CR retards the age-associated accumulation of oxidatively damaged molecules in rodents; the reader can find a review of many of these studies in Yu (1996). There is also some evidence that CR acts the same way in monkeys (Zainal et al., 2000). This attenuation of the accumulation of oxidative damage must be due to either a decreased rate of generation of reactive oxygen molecules, or to increased efficiency of protective processes, or to an increase in repair activity, or to a combination of these processes. While several studies have showed that CR decreases the formation of reactive oxygen molecules by isolated mitochondria and microsomes from CR rodents, Feuers et al. (1993) point out that little is known about this action in a functioning intact rat or mouse. Of course, it is risky to draw physiologically relevant conclusions solely from in vitro studies. Although many studies have shown that CR increases the activity or retards the age-associated decrease in activity of enzymes like catalase, superoxide dismutase, and glutathione peroxidase, which protect rodents from oxidative damage, other studies have shown just the opposite effect (for examples see Richardson, 1991; Luhtalta et al., 1994). Obviously, the action of CR on the antioxidant enzymes is far from simple.

The ability of CR to bolster the non-enzymatic antioxidant defenses, such as increasing the levels of reduced glutathione, has been clearly shown (Armeni et al., 1998). CR is also known to enhance the ability to repair oxidatively damaged DNA (Guo et al., 1998) and to replace damaged proteins (Lewis et al., 1985).

Thus, it seems beyond question that CR protects rodents from damage caused by oxidative stress. Is this action the major mechanism underlying the life-prolonging and senescence-retarding actions of CR? The answer to this question depends on whether oxidative stress plays a major role in ageing. There is a body of evidence in support of a major role for oxidative damage in ageing: the classic study of Arking et al. (1991) showed that flies selected for postponed senescence have an increased resistance to oxidative stress. Also, the study of Migliaccio et al.

(1999) showed that mice with a homozygous mutation in the $p66^{shc}$ gene exhibit a markedly increased life span and an enhanced resistance to oxidative stress. Female mice heterozygous for the disruption of the IGF-1 receptor gene also have an extended life span and increased resistance to oxidative stress (Holzenberger et al., 2003).

However, there also have been studies indicating, at least in some instances, that oxidative stress is not an important factor in the occurrence of senescence. Hauck et al. (2002), reporting work on growth hormone receptor/binding protein gene knockout mice, found that although there is an increase in life span compared to the wild type, the knockout mice are more susceptible to damage from paraquat. Van Remmen et al. (2003) found that in mice deficient in Mn-superoxide dismutase, the increased oxidative stress/damage does not affect life span and other age-sensitive parameters. Orr et al. (2003) reported that the life span of long-lived *Drosophila* is not increased by the over-expression of the following antioxidant enzymes: CuZn-superoxide dismutase, Mn-superoxide dismutase, thioredoxin reductase, and catalase. Thus, it remains an open question whether CR's ability to attenuate oxidative damage plays a major role in its life-extending action.

3.5. Altered Glucose–Insulin System Hypothesis

Although by 1990, it had been known for some time that fasting levels of plasma glucose and insulin are lower in rodents on a CR regimen, the question had not been addressed as to whether such is the case under usual daily living over a lifetime. Therefore, a lifetime longitudinal study on male F344 rats was carried out in our laboratory (Masoro et al., 1992). We found that throughout the lifetime, CR decreased the mean 24-h plasma glucose concentration by about 15 mg/dl and the insulin concentration by about 50%. Moreover, the rats on the CR regime used glucose as fuel at the same rate per unit of metabolic mass as did the rats fed ad libitum, despite the lower plasma glucose and markedly lower plasma insulin levels. We concluded that CR either increased glucose effectiveness or insulin responsiveness or both, and proposed that the lifetime maintenance of low levels of glucose and markedly low levels of insulin played a major role in the life-extending and related actions of CR (*Altered Glucose–Insulin System Hypothesis*).

CR has been found to reduce plasma glucose and insulin concentrations in fasting rhesus monkeys (Kemnitz et al., 1994; Lane et al., 1995). In addition, CR increases insulin sensitivity in rhesus and cynomolgus monkeys (Kemnitz et al., 1994; Bodkin et al., 1995; Lane et al., 1995; Cefalu et al., 1997).

In recent years, the focus has been on the insulin component of the glucose–insulin system. The major reasons for this emphasis are findings that loss-of-function mutations of the insulin signaling system result in life extension in three species: *C. elegans* (Kenyon et al., 1993; Wolkow et al., 2000), *D. melanogaster* (Clancy et al., 2001; Tatar et al., 2001), and mice (Blüher et al., 2003). Clearly, by maintaining plasma insulin at a markedly low level throughout life, CR is in effect decreasing insulin signaling.

3.6. Alteration of the Growth Hormone–IGF-1 Axis Hypothesis

In the early 1990s, Bill Sonntag's group reported that CR results in markedly lower levels of plasma insulin-like growth factor-1 (IGF-1) in rats and mice (Breese et al., 1991; Sonntag et al., 1992; D'Costa et al., 1993). The possible significance of this finding was not recognized until some years later when the Ames and Snell Dwarf mice were found to exhibit life extension (Brown-Borg et al., 1996; Bartke et al., 2001; Flurkey et al., 2001). Among other endocrine characteristics, these dwarf mice exhibit an inability to secrete growth hormone and consequently low plasma levels of IGF-1. Furthermore, Coschigano et al. (2000) have studied mice in which the growth hormone receptor/binding protein gene has been disrupted, and found that they exhibit high levels of plasma growth hormone, very low levels of plasma IGF-1, and life extension. These and related studies led to the view that the reduction of plasma IGF-1 in rodents on CR regimens may play an important role in its life-extending action, which I term the *Alteration of the Growth Hormone–IGF-1 Axis Hypothesis*.

Further support for the foregoing hypothesis comes from the fact that nematodes and fruit flies do not have separate receptors for insulin and IGF-1. Thus, the genetic studies that implicate reduction in insulin signaling in the life extension of these species also apply to IGF-1 signaling.

Holzenberger et al. (2003) have reported findings that at first sight appear to provide direct support for the role of the attenuating IGF-1 signaling in life extension of mice. They studied male and female mice heterozygous for the disruption of IGF-1 receptor gene and found that the females with this disruption have a statistically significant increase in mean length of life compared to wild type females, while in the case of the male mice, the increase was not statistically significant. However, there are several concerns about this study and its use to draw conclusions about the role of decreased IGF-1 signaling in the actions of CR. First, since only a small number of mice was studied, to conclude that reduction in IGF-1 signaling increases longevity in the female but not in the male is a precarious claim. Second, if such a gender difference were truly the case, it would indicate that the reduction in IGF-1 signaling does not play an important role in life extension by CR, because it has been found that CR increases the length of life in a variety of rat and mouse strains with no evidence that the extent of the effect relates to gender (Lewis et al., 1999). In addition, there are other issues that are not addressed in the Holzenberger et al. paper. The wild type 129/Sv strain of mice used in this study had a much shorter life (mean length of life less than 19 months in the females) than most mice strains, and it is not clear from the paper whether this is

typical of this mouse strain. In fact, while the mice were maintained in a conventional facility (rather than in specific-pathogen-free facility), the problem of infectious disease was neither discussed, nor were any pathology data presented. Indeed, it seems essential to know whether the life extension relates to an effect on a specific disease process in the female mice.

3.7. Hormesis Hypothesis

Hormesis refers to the phenomenon whereby a usually detrimental environmental agent (radiation, chemical substance, etc.) changes its role to provide beneficial effects when administered at low intensities or concentrations (Furst, 1987). In regard to biological gerontology, I have modified the definition somewhat as follows: hormesis is the beneficial action resulting from the response of an organism to a low-intensity stressor. These beneficial actions include: increased longevity, retardation of senescent deterioration, retardation of age-associated diseases, and enhanced coping with intense stressors. I proposed that CR's ability to do all of these is due to hormesis and termed it the *Hormesis Hypothesis* (Masoro, 1998).

The first issue that needs to be addressed is whether CR is a low-intensity stressor. Strong support comes from findings in both rats and mice that CR results in the daily elevation of circadian peak plasma free corticosterone levels throughout the life span (Sabatino et al., 1991; Han et al., 1995).

The next issue is whether rodents on a CR regimen exhibit an enhanced ability to cope with intense stressors. Indeed, CR has been found to have beneficial actions in this regard. CR attenuates the loss in body weight of rats following surgery for the implantation of jugular cannulae (Masoro, 1998), and it increases the ability of rats to survive intense heat stress (Heydari et al., 1993). The inflammatory reaction following the injection of carageenan into the footpad is attenuated in mice on a CR regimen (Klebanov et al., 1995). CR also protects rodents from the damaging action of toxic drugs (Berg et al., 1994; Duffy et al., 1995; Keenan et al., 1997).

Does CR's ability to increase the resistance of rodents to acute, intense stressors have any relevance to its ability to retard senescence and extend life? The *Disposable Soma Hypothesis of Ageing* poses that less energy is used for somatic maintenance than needed for indefinite survival (Kirkwood, 1977). Thus, with increasing age, there is an accumulation of damage caused by both endogenous stressors, such as the reactive oxygen molecules produced during fuel utilization, and a spectrum of environmental factors, such as chemical toxins, infectious agents, etc. By promoting the hormetic processes, it is proposed that CR attenuates the rate of accumulation of damage from these various agents, thereby retarding senescent deterioration and extending life.

What are the organismic, cellular, and molecular mechanisms involved in the hormetic action of CR? At an organismic level, the daily elevation of blood glucocorticoids could well be one. It is well known that glucocorticoids play a key role in enabling mammalian species to cope with stressors (Munck et al., 1984). Indeed, Chung et al. (2001) proposed the *Inflammation Hypothesis of Aging*, which postulates that inflammatory processes play a key role in aging. The daily moderate elevation of plasma free corticosterone induced by CR in rats and mice would be expected to have a significant anti-inflammatory action.

At the cellular and molecular level, another possibility is an increase in the activity of genes that protect cells from the damaging action of harmful agents (Papaconstantinou et al., 1996). Indeed, CR has been shown to increase the induction of hepatic HSP 70, one of these protective proteins, in response to heat stress (Heydari et al., 1993).

Finally, studies of *S. cervisiae* by Anderson et al. (2003) detailed the afferent hormetic pathway by which CR extends the replicative life of this yeast species. They found that not only is a functional *Sir2* gene needed for CR-induced replicative life extension but, in addition, a functional *PNC1* gene is required. The *PNC1* gene encodes a protein with nicotinamidase activity and CR acts to increase the amount of this enzyme. The deacetylase activity of the SIR2 protein involves the generation of nicotinamide, which is an inhibitor of the deacetylase activity of SIR2 protein. By reducing the level of nicotinamide, the PNC1 protein increases the deacetylase activity of the SIR2 protein, and it is this deacetylase activity that plays a key role in the CR-induced increase in the replicative life span of this yeast species. They also found that this same pathway is involved in the heat-stress- and osmotic-stress-induced extension of replicative life span of *S. cervisiae*. Thus, a spectrum of low-intensity stressors shares a common hormetic pathway. Moreover, as discussed above, there is growing evidence that sirtuin proteins are also involved in the life-extending action of CR in other species, including mammals.

These recent findings provide strong support for the *Hormesis Hypothesis*, a concept that embraces many of the other proposed hypotheses. Indeed, many of those hypotheses, such as the *Oxidative Damage Attenuation Hypothesis*, appear to be merely specific components of the hormetic mechanism underlying life extension by CR. However, there is concern that although many low-intensity stressors increase both mean and maximum length of life in many species, some apparent stressors do not. For example, Holloszy et al. (1985) found that voluntary exercise increased the mean but not the maximum length of life of rats. In still another study, Holloszy and Smith (1986) reported that cold exposure increased neither mean nor maximum length of life of rats. The explanation may simply be that hormesis is a response to low-intensity stressors and that high-intensity stressors are usually detrimental rather than beneficial. Indeed, it is not evident that voluntary exercise is a stressor for rats, while Holloszy and Smith's cold

exposure protocol may have been too intense to induce hormesis.

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