

Toward a unified theory of caloric restriction and longevity regulation

David A. Sinclair*

Department of Pathology, Harvard Medical School, 77 Avenue Louis Pasteur, Boston, MA 02115, USA

Received 22 November 2004; received in revised form 3 February 2005; accepted 15 March 2005

Available online 11 May 2005

Abstract

The diet known as caloric restriction (CR) is the most reproducible way to extend the lifespan of mammals. Many of the early hypotheses to explain this effect were based on it being a passive alteration in metabolism. Yet, recent data from yeast, worms, flies, and mammals support the idea that CR is not simply a passive effect but an active, highly conserved stress response that evolved early in life's history to increase an organism's chance of surviving adversity. This perspective updates the evidence for and against the various hypotheses of CR, and concludes that many of them can be synthesized into a single, unifying hypothesis. This has important implications for how we might develop novel medicines that can harness these newly discovered innate mechanisms of disease resistance and survival.

© 2005 Elsevier Ireland Ltd. All rights reserved.

Keywords: Histone deacetylase; SIR2; PBEF; NAMPT; Visfatin; Aging; Sirtuin; HDAC; Nicotinamide; Resveratrol; Xenohormesis

Throughout history, numerous societies have touted the health benefits of food limitation, including the Ancient Greeks and Romans (Dehmelt, 2004). In modern times, Professor Maurice Gueniot, President of the Paris Medical Academy at the turn of the 20th century, is famed for living on a restricted diet and for dying at the age of 102. The first widely recognized scientific study of restricted diets and their ability to extend lifespan was published in 1935, by McCay and co-workers, coincidentally the year of Maurice Gueniot's death. The researchers found that feeding rats with a diet containing 20% indigestible cellulose dramatically extended mean and maximum lifespan (McCay et al., 1935). Variations of this dietary regimen, now known as caloric restriction (CR) or dietary restriction (DR), are the most effective way of extending the maximum lifespan of mammals without genetically altering them (Masoro, 2000).

Over the past 70 years, there have been at least 10 plausible theories to explain how CR works but almost all of them have fallen out of favor because of contradicting data (Fig. 1). Of those that remain, many fail to explain the myriad observations about CR and lifespan-extension in general. It would seem that CR is too complex ever to be

described by a single hypothesis, and the development of drugs that might mimic CR's beneficial effects seems, to many researchers, a distant, possibly unachievable goal.

There is, however, reason for renewed hope. In the past few years, a hypothesis has gained considerable support from a number of laboratories studying different aspects of CR and lifespan-extension (Calabrese, 2004; Mattson et al., 2002b; Rattan, 2004b; Sinclair, 1999; Turturro et al., 2000). The so-called Hormesis Hypothesis of CR is a different way of thinking about the problem that could breath new life into the CR field by uniting numerous, seemingly unrelated observations in a variety of species (Fig. 2). In essence, the hypothesis states that low caloric intake is a mildly stressful condition that provokes a survival response within the organism, helping it to survive adversity by altering metabolism and increasing the organism's defenses against the causes of aging, whatever they may be. This view of CR has led to another theory to explain why a class of small polyphenolic molecules produced by stressed plants can directly modulate stress-response pathways in yeast, worms, and flies. The Xenohormesis Hypothesis states that organisms have evolved to pick up on stress-signaling molecules from other species in their environment because it allows them to shift into a survival mode in advance of an environmental decline (Howitz et al., 2003; Lamming et al.,

* Tel.: +1 617 432 3931; fax: +1 617 432 1313.

E-mail address: david_sinclair@hms.harvard.edu.

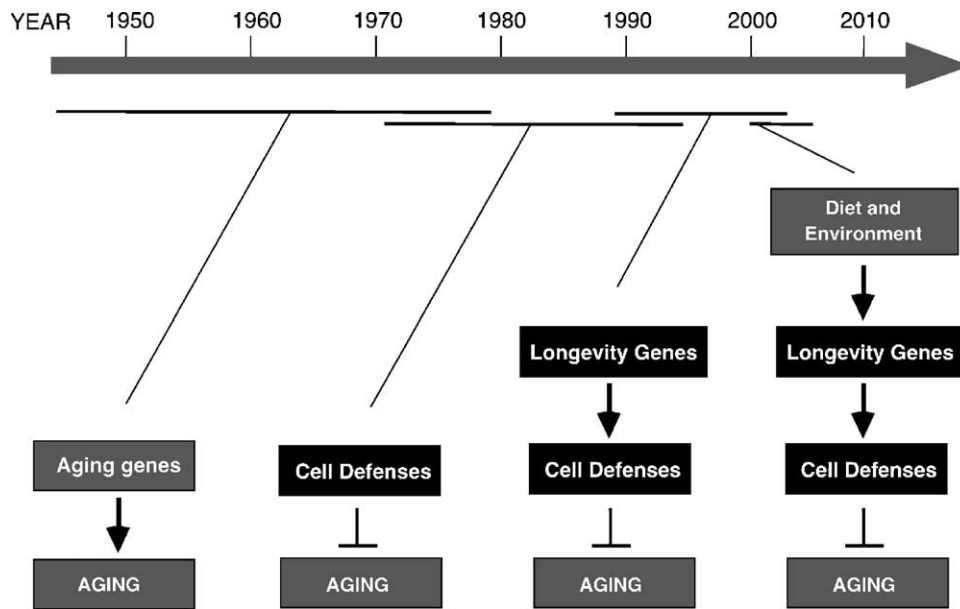


Fig. 1. Changing views about aging. Before the 1970s, the predominant view was that aging is caused by “death” genes that directed the process as if it were an extension of development. Evolutionary biologists argued that aging is not adaptive for most species, and this idea was laid to rest. After that, the focus was on the causes of aging and the mechanisms that defend cells against them. During the late 1980s and 1990s, a number of genetic screens in simple organisms such as yeast, worms, and flies identified single gene mutations that could dramatically extend lifespan. Single gene mutations in long-lived dwarf mice were also characterized. Then, around the turn of the century, it was shown that some of these genes are activated by low caloric intake and other mild biological stresses, consistent with the hypothesis that these genes had evolved to protect organisms during times of adversity, and that their chronic activation leads to increased health and lifespan.

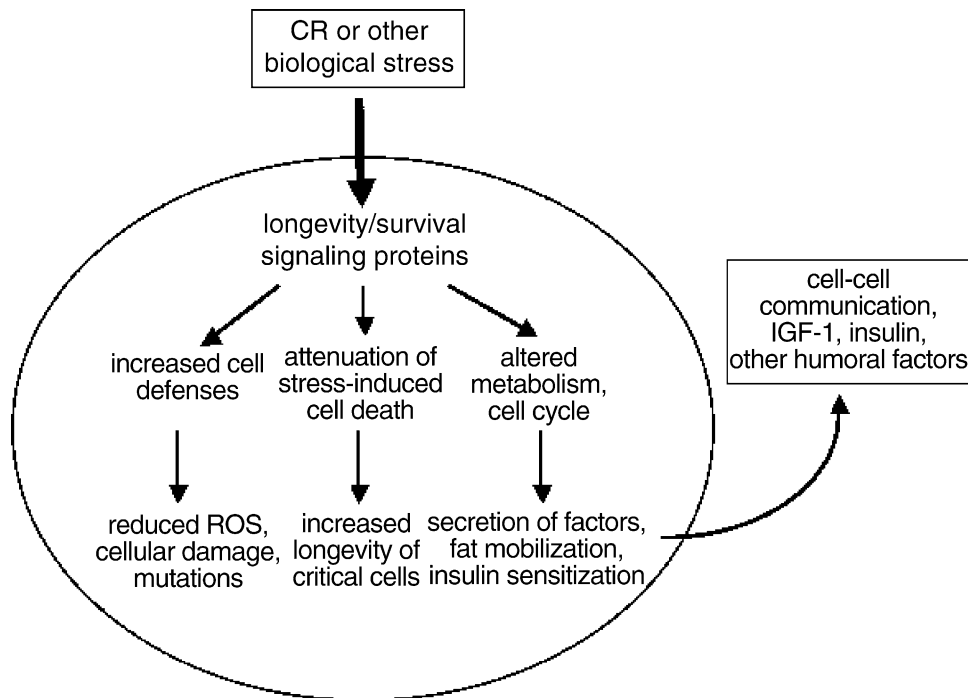


Fig. 2. The Hormesis Hypothesis of CR. The theory states that CR is a mild stress that provokes a survival response in the organism, which boosts resistance to stress and counteracts the causes of aging. The theory unites previously disparate observations about ROS defenses, apoptosis, metabolic changes, stress resistance, and hormonal changes and is rapidly becoming accepted as the best explanation for the effects of CR.

2004). Xenohormetic molecules are seen as one possible path to a new class of medicines that work by stimulating our innate defenses against disease.

1. Early hypotheses of CR

1.1. Developmental delay

Originally, CR was thought to extend lifespan by slowing the growth and development of the animal (McCay et al., 1935) (Table 1). This idea made perfect sense because, at that time, aging was widely believed to be governed by genes that caused aging, in the same way as development is. In fact, testing this hypothesis was the reason for McCay's original experiments with CR, and he got the result he was expecting (McCay, 1935; McCay et al., 1935, 1975). Unfortunately for McCay, the hypothesis did not survive long. Researchers quickly showed that placing adults on a CR diet late in life also extended lifespan and today the idea that aging is caused by a genetic program is no longer considered valid by the majority of researchers in the field (Krystal and Yu, 1994). Instead, aging is thought to occur because organisms lack sufficient energy to fully prevent and repair cellular damage – due to the competing demands of other activities such as growth and reproduction – and because genes that favor health and fecundity in youth can be maintained in a population even though they are deleterious to older individuals (Williams, 1957; Charlesworth, 2000; Kirkwood and Holliday, 1979; Medawar, 1946).

1.2. Reduced metabolic rate

German physiologist Rubner (1908) reported that animals of various sizes utilize a similar number of calories per weight per lifetime. This gave rise to Pearl's "Rate of Living Hypothesis" (Pearl, 1928). Between 1950 and 1981, four groups showed that reducing the food intake of rats slows metabolic rate per unit of body mass implying that CR works by lowering metabolic rate (reviewed in Krystal and Yu, 1994). However, numerous other studies, many of them from Masoro and co-workers, have shown that CR animals have equal or higher metabolic rates than AL animals after they experience an initial drop in metabolic rate in the first six weeks of the diet (Masoro, 1998; Masoro et al., 1982; McCarter et al., 1985; Speakman et al., 2004, 2003; Yu et al., 1985).

Most studies using simple eukaryotes as models for CR have also failed to find evidence for the metabolic rate theory. For example, studies by Vanfleteren and co-workers studying *C. elegans* found no evidence that CR decreases metabolic rate (Braeckman et al., 2002) and in one study they observed an increase (Houthoofd et al., 2002). Partridge and co-workers also found no positive correlation between diet and metabolic rate for *D. melanogaster* (Braeckman et al., 2002; Hulbert et al., 2004). In summary, although the metabolic rate hypothesis in its simple form is attractive, it is

fair to say that the data on the relationship between metabolic rate and lifespan are contradictory and the theory is no longer considered valid in its simple form (Speakman et al., 2003).

There is a variation on this theme, sometimes referred to as the "Uncoupling to Survive Hypothesis", which states that CR reduces the efficiency of the mitochondrial electron transport chain, which reduces the production of reactive oxygen species (ROS). This hypothesis deserves serious consideration and will be discussed in a separate section below.

1.3. Laboratory gluttons

Numerous researchers have speculated that CR might be a laboratory artifact. The argument is as follows: CR is the sort of diet an animal would have in the wild, and the ad libitum controls in CR experiments might simply be grossly overfed, leading to ill health (Cutler, 1982; Hayflick, 1994). Although this argument is frequently raised, it is not supported by evidence. For a start, many laboratories have switched to a controlled diet instead of feeding animal ad libitum, in part to ensure that they are not overfed. These laboratories report that CR still increases lifespan to the same extent as those employing ad libitum controls (Pugh et al., 1999). Another argument in favor of CR is that it works on almost every species it has been tested on including yeast, worms, flies, fish, dogs, and spiders (Masoro, 2000). In the same vein, yeast and flies often have access to more food than is typically supplied under laboratory conditions. Rodents in their native environment must eat more than the CR animals for at least part of their life, otherwise they would be infertile and the species would become extinct (Austad, 2001). In an elegant perspective covering this issue, Austad calculated that the amount of food consumed by a typical laboratory mouse is similar to that consumed by one in the wild (~3 kJ/(g day)) and concluded that CR mice are "not grossly overfed" (Austad, 2001).

2. Current hypotheses of CR: pre-synthesis

2.1. Glucocorticoid cascade

The "glucocorticoid cascade hypothesis of aging" proposes that glucocorticoids, which play a critical role in the body's stress response, are a cause of aging, and by extension, CR works by attenuating glucocorticoid pathways (Masoro, 1996; Nelson et al., 1995; Spindler et al., 1991). Although this hypothesis is not discounted, a number of studies have reported that CR animals have higher levels of a key glucocorticoid, corticosterone, and there is no increase in this steroid in older animals, contrary to the prediction of the hypothesis (reviewed in Krystal and Yu, 1994). These results present a serious challenge to the hypothesis but clearly more work is needed in this area to resolve the issue.

2.2. Decreased fat

Given the negative effects of obesity, it is tempting to assume that CR provides health benefits simply by keeping fat stores low (Berg and Simms, 1960). There is little doubt that fat stores and the hormones they secrete play a role in lifespan determination (Bluher et al., 2003; Tatar et al., 2003). But the relationship between CR and fat is not simple. Despite early indications that fat content and lifespan inversely correlate (Bertrand et al., 1980), over the past 20 years this correlation has broken down in many studies (Harrison et al., 1984; Masoro, 1995). A recent study of dogs found no relationship between lifespan and body mass, fat mass, or lean body mass (Speakman et al., 2003). In the case of rodents, those that maintain a higher level of fat are often longer lived (Masoro, 1995).

The ‘decreased fat hypothesis’ has received new life following the demonstration that fat cells secrete factors that seem to promote aging when present in excess (Barzilai and Gabriely, 2001; Barzilai and Gupta, 1999; Gupta et al., 2000) and that a fat-specific knockout of the insulin receptor extends mouse lifespan (Bluher et al., 2003). While it is likely that fat cells play a role in modulating the pace of aging by secreting humoral factors, and this is a highly relevant aspect of longevity research, there is little evidence to support the simple version of the hypothesis: that reduced fat content is what extends maximum lifespan in CR animals.

2.3. Reduced reactive oxygen species

A variety of metabolic reactions and exogenous agents generate ROS that can damage cellular constituents such as proteins, DNA, and lipids. Because our cellular defense and repair systems are not infinitely efficient, a surprising

amount of ROS-mediated damage occurs daily within our cells. Some of this damage is not (and cannot) be reversed, and thus accumulates over time, possibly underlying the aging process. Consistent with this idea, older animals contain higher quantities of oxidized lipids and proteins, as well as damaged/mutated DNA, particularly in the mitochondrial genome (reviewed in Droge, 2003; Dufour and Larsson, 2004). Strikingly, mice with an accelerated rate of mutation in mitochondrial DNA exhibit signs of premature aging such as weight loss, reduced subcutaneous fat, hair loss, osteoporosis, anemia, and reduced fertility, which has been regarded by some as strong evidence for the mitochondrial DNA damage hypothesis of aging (Trifunovic et al., 2004).

With regards to CR, restricted animals have less ROS-mediated damage, including lipid peroxidation and loss of membrane fluidity, oxidatively damaged proteins (specifically “carbonylated” proteins), and oxidative damage to DNA (reviewed in Barja, 2004; Merry, 2002). It is worth noting that, even among the scientists who hypothesize that ROS are relevant to CR, there is some debate as to whether the diet works primarily by decreasing ROS *production* or increasing ROS *defenses* and *repair*. For a summary of the myriad data on this topic, an excellent review has recently been published (Merry, 2004). In this general review, suffice it to say that there are supportive data for both mechanisms, and they are by no means mutually exclusive (Table 1).

Although the free-radical theory of CR remains popular, many believe the experimental evidence is not yet convincing (Lindsay, 1999). Proof that anti-oxidants are beneficial is mainly limited to the demonstration that they slightly increase average life span in rodents and flies. In *Drosophila*, there has been considerable work done to test this hypothesis but the data are contradictory. Flies transgenically altered to overexpress human SOD are stress

Table 1
Prominent hypotheses to explain lifespan extension by CR

Hypothesis	Evidence for	Evidence against
Developmental delay	CR slows development, animals with slower development tend to live longer	CR works post-puberty
Altered energy metabolism	CR lowers body temperature, some evidence for lowering of mutation frequency. Good evidence for uncoupling of oxidative phosphorylation, thus reducing ROS	CR does not lower metabolic rate or frequency of certain mutations in mitochondria
Endocrinological changes	CR alters numerous endocrine factors; mice mutant for IGF-1/growth hormone live longer	Glucose levels not significantly lowered by CR; IGF-1 mutant mice still respond to DR. Only partial overlap between dwarf and CR mice
Enhanced cell defenses/decreased apoptosis	Cells from CR and long-lived animals tend to be stress resistant and resistant to apoptosis, especially cells that are difficult to replace	CR increases rates of cell death in some tissues such as liver
Hormesis, i.e., CR provokes a mild stress response causing enhanced cell defenses, coordinated by endocrine system	CR proven to work via hormesis for budding yeast; long lived animals have increased stress resistance; fits with evolutionary theories of aging	Hormesis is unproven in mammals

resistant and live up to 40% longer (Parkes et al., 1998; Reveillaud et al., 1992; Spencer et al., 2003), but the effect is genotype- and sex-specific (Spencer et al., 2003). Molecules that might soak up ROS such as melatonin, carnosine, epitalamin (a pineal peptide), and epitalon (a short peptide of Ala–Glu–Asp–Gly) increase average lifespan of flies up to 16% (Izmaylov and Obukhova, 1999; Khavinson et al., 2000; Yuneva et al., 2002) but whether these effects are due to the molecules' anti-oxidant properties, or some other effect such as hormesis (see below), is not yet clear. Contradicting the hypothesis are studies showing no correlations between fly lifespan or CR and decreased ROS production (Barja, 2004; Merry, 2002; Miwa et al., 2004).

In mammals, the situation is just as muddy. A recent study of rat liver and brain reported that CR does not affect the accumulation of a common age-related deletion in mitochondrial DNA (Cassano et al., 2004). There have also been numerous reports that CR has little effect on ROS defense mechanisms and, if anything, CR attenuates an age-dependent increase (Gong et al., 1997; Guo et al., 2001; Luhtala et al., 1994; Rojas et al., 1993; Stuart et al., 2004). This discordance could be due to the fact that many of the studies examined different tissues, or perhaps it is because some measured mRNA levels and others protein activity, making direct comparisons between studies difficult. That aside, perhaps the most convincing evidence against the free-radical theory of aging is that mice lacking superoxide dismutase (SOD2) incur greatly increased oxidative damage, yet they do not show signs of premature aging by a variety of measures (Van Remmen et al., 2003). In summary, there is considerable disagreement within the field about the validity of the free-radical damage hypothesis, and the debate looks as though it will continue.

2.4. Cell Survival Hypothesis

Aging is generally associated with increased rates of stress-induced apoptosis. The cumulative effects of cell loss have been implicated in various diseases including neurodegeneration, retinal degeneration, cardiovascular disease, and frailty (reviewed in Zhang and Herman, 2002). The 'Cell Survival Hypothesis of CR' states that the increased lifespan is due to an attenuation of cell loss, particularly those cells which cannot easily be replaced such as neurons and stem cells (Cohen et al., 2004b; Koubova and Guarente, 2003). Consistent with this idea, cells cultured from long-lived genetic mutants such as the p66^{sch} knockout mouse and long-lived dwarfs are typically less prone to stress-induced apoptosis (Migliaccio et al., 1999; Murakami et al., 2003).

Rates of apoptosis in cells and tissues from CR animals have been examined and results vary depending on the tissue. Many studies have reported that CR increases rates of apoptosis or genes that promote apoptosis, especially in rapidly dividing tissues such as skin, pre-neoplastic cells,

and the immune system (Cao et al., 2001; Mukherjee et al., 2004; Tsuchiya et al., 2004; Wachsman, 1996). This increase in apoptosis is thought to be a major mechanism by which CR rats suppress cancer and maintain the health of dividing tissues (James et al., 1998; Zhang and Herman, 2002).

In contrast, a number of recent studies indicate that CR protects a variety of cell types from apoptosis including neurons, kidney, liver, and certain immune cells (Calingasan and Gibson, 2000; Hiona and Leeuwenburgh, 2004; Lee et al., 2004; Monti and Contestabile, 2003; Selman et al., 2003). This literature is extensive and covered elsewhere in more detail (Howitz and Sinclair, 2005, for example). Recent highlights include the findings that CR protects hippocampal neurons from apoptosis in a mouse model of Alzheimer disease (Mattson et al., 2002b) and that neurons of CR animals express high levels of two key apoptosis inhibitors and are more resistant to stress-induced apoptosis (Hiona and Leeuwenburgh, 2004; Shelke and Leeuwenburgh, 2003). Hepatocytes from CR animals are also less susceptible to cytotoxins and genotoxins (Shaddock et al., 1995), in part because expressions of p53, gadd153/chop, and Fas receptor are repressed (Ando et al., 2002; Hatano et al., 2004; Ikeyama et al., 2003; Tanaka et al., 2004).

A recent study showed that in CR rats, levels of the SIRT1 protein – a homolog of the yeast Sir2 longevity factor discussed below – are elevated, leading to decreased apoptosis (Cohen et al., 2004b). SIRT1 attenuates stress-induced apoptosis by deacetylating both p53 (Motta et al., 2004; Vaziri et al., 2001) and the Ku70 protein. Deacetylated Ku70 sequesters the pro-apoptotic protein Bax, preventing it from initiating, mitochondrial-driven apoptotic cascades (Cohen et al., 2004b). SIRT1 also deacetylates the FOXO3 transcription factor, tipping the scales further toward cell protection and survival (Antebi, 2004; Brunet et al., 2004). It has been proposed that SIRT1 serves to protect irreplaceable cells such as neurons and stem cells from death during times of stress, thus maintaining physiological function with age (Cohen et al., 2004b). In summary, it is now well accepted that CR modulates a cell's susceptibility to apoptosis. Whether it is an increase or decrease in apoptosis seems to depend on the cell type and stimulus.

2.5. Protein turnover

Aging is associated with a variety of protein modifications including carbonylation, glycation, racemization, isomerization, and deamination (Stadtman, 1995). Protein turnover is an efficient way to maintain functional proteins. There is abundant evidence from gene expression profiling and biochemical studies in *C. elegans* and mammals, that protein turnover rates and autophagic processes (i.e., auto-digestion) decline with age and that this decline is attenuated by CR (Del Roso et al., 2003; Lewis et al., 1985; Tavernarakis and Driscoll, 2002). Whether or not this is a cause or a symptom of aging is not yet clear but it is an area of growing interest.

2.6. Decreased glucose and insulin levels

Diabetes mellitus or type II diabetes is characterized by a decrease in the sensitivity of tissues to insulin and a correspondingly high level of serum glucose. High glucose levels lead to cellular alterations more often seen in the elderly, including the accumulation of advanced glycation end-products (AGE). Many organs and tissues of type II diabetic individuals tend to age faster than normal. One way CR might improve health and possibly extend lifespan is by improving insulin sensitivity and keeping blood glucose levels down (reviewed in Kalant et al., 1988). Consistent with this idea, CR animals have slightly lower blood glucose and dramatically lower insulin levels (Kalant et al., 1988; Masoro, 1992). They are also relatively resistant to diabetes and cardiovascular disease (Weindruch and Walford, 1988). The reduction in circulating glucose by CR is due to increased glucose uptake in skeletal muscle and fat pads as a result of GLUT-4 localizing to the plasma membrane (Cartee et al., 1994; Dean et al., 1998a,b); the decrease in insulin is attributable to a lower rate of secretion from pancreatic β -cells (Dean et al., 1998b). While these effects are clearly associated with CR, it remains to be seen if low glucose and insulin levels are an actual cause of the lifespan increase. Certainly, the theory struggles to explain how a reduced level of insulin and/or glucose alone could give rise to the multitude of effects seen in CR animals.

2.7. Other endocrinological changes

It has been known for many years that CR alters the levels of key hormones, and that some of these changes are associated with increased longevity. There is now good evidence that these changes actually contribute to the longevity of the animals (Bartke et al., 2001a; Tatar et al., 2003). Spontaneous genetic alterations in mice that lead to extensions in lifespan are associated with profound alterations in hormonal levels. For example, the Ames and Snell dwarf mice have mutations in *prop-1* and *pit-1*, respectively, which cause stunting of the anterior pituitary and the animals are deficient in a number of endocrine factors including growth hormone (GH), prolactin and thyroid stimulating hormone (TSH) (reviewed in Bartke et al., 2001b; Miller et al., 2002).

Although there is little doubt that endocrinological factors can influence lifespan, whether or not these animals are models for CR is the subject of debate (Bluher et al., 2003; Masternak et al., 2004; Miller et al., 2002; Mobbs et al., 2001). While it is true that IGF-1 and insulin levels are lower in both CR animals and in dwarf mice, Bartke et al. (2001b) have reported that the lifespan of the dwarf can be further increased by CR, indicating that they might rely on alternative mechanisms, at least in part. This idea is supported by comparing the gene expression profiles of CR and GH knockout mice (Masternak et al., 2004; Miller et al., 2002; Tsuchiya et al., 2004). Similar conclusions have been

reached in the *C. elegans* field. Vanfleteren and co-workers provided good evidence that mild CR does not involve IGF-1 signaling but that intense CR or starvation does, and these two mechanisms have an additive effect on lifespan (Houthoofd et al., 2003). In summary, it is clear that the endocrine system is important in determining longevity and that certain hormones such as IGF-1 seem to play an important role in coordinating a systemic response to CR. Even so, the underlying intracellular basis of the increased lifespan of CR animals remain a mystery.

3. The Hormesis Hypothesis and stress-responsive survival pathways: a synthesis

A positive correlation between mild biological stress and increased longevity has been known for decades in the *Drosophila* aging field (Rose et al., 1992; Smith, 1958; Strehler, 1967; Westerman and Parsons, 1972) and over the past few years researchers have proposed that these early observations might be related to CR. The formal synthesis of these ideas is known as “the Hormesis Hypothesis of CR” (Anderson et al., 2003; Lithgow, 2001; Masoro, 2000; Masoro and Austad, 1996; Mattson et al., 2002a; Rattan, 1998, 2004a; Turturro et al., 1998, 2000). The hypothesis proposes that CR imposes a low-intensity biological stress on the organism, and that this elicits a defense response that helps protect it against the causes of aging. The term “hormesis” refers to beneficial actions resulting from the response of an organism to a low-intensity stressor (Calabrese, 2004; Calabrese et al., 1987; Southam and Ehrlich, 1943). The theory is a radical departure from earlier ones because it is based on the premise that CR is due to an *active* defensive response of the organism, as opposed to *passive* mechanisms. The theory has been recently expanded by Sinclair and Howitz to include the idea that organisms can pick up on chemical stress cues from other species under stress or CR, either in their food or environment, and use these to activate their own defense pathways in preparation for adverse conditions (Howitz et al., 2003; Lamming et al., 2004). This idea, known as “the Xenohormesis Hypothesis”, is discussed below.

The Hormesis Hypothesis of CR makes four key predictions:

1. CR induces intracellular cell-autonomous signaling pathways that respond to biological stress and low nutrition.
2. The pathways in (1) help defend cells and tissues against the causes of aging.
3. The pathways in (1) regulate glucose, fat, and protein metabolism in a way that enhances the chance of survival during times of stress.
4. The pathways in (1) are under the control of the endocrine system to ensure that the organism acts in a coordinated fashion.

One of the most encouraging aspects of the Hormesis Hypothesis is that it explains so many different observations about CR, ranging from the ability of CR animals and their individual cells to withstand stress, to the finding that lower organisms have a conserved stress-response pathway that affects lifespan, to the link between the CR and the endocrine system. It could be argued that many of the current CR hypotheses have stemmed from researchers attempting to describe different parts of the same phenomenon. Below are presented the key findings that led to – and have lent credence to – the Hormesis Hypothesis.

3.1. Single genes regulate lifespan

Only 20 years ago, few researchers suspected the existence of single genes that could regulate lifespan. This was based in part on the fact that aging is an incredibly complex process, involving numerous tissues and systems and impacted by thousands of genes. But when single gene mutations were uncovered in mice, then worms and yeast, that could dramatically extend lifespan (D’Mello et al., 1994; Friedman and Johnson, 1988; Kenyon et al., 1993), it was clear that certain views about aging were in need of revision.¹

Around the same time, evolutionary biologists were formulating ideas that were consistent with there being a few key regulatory genes for aging. Holliday (1989) first proposed that the effect of CR is an evolutionary adaptation that allows organisms to survive periods of low food availability. Masoro and Austad (1996) then expanded this idea, merging it with the ideas of Kirkwood and Calbrese. The synthesis of these theories is difficult to summarize but essentially includes the idea that every individual has access to limited resources (i.e., energy supply) and that these resources can only be allocated to a finite number of activities, the two primary ones being reproduction and somatic (body) maintenance (Kirkwood and Holliday, 1979). During times of adversity such as low food supply, organisms are able to divert more of their resources to maintaining their soma, at the expense of growth and reproduction, until conditions improve. Today, there is general consensus among many of the leaders in the CR field that the health benefits of CR very likely derive from an organism’s defense response to a perceived threat to its survival. The complete Hormesis Hypothesis needs to include the idea that key longevity genes control this allocation of resources, and that single gene mutations lock the organism into constitutively allocating more resources to somatic maintenance and survival.

¹ The term “gerontogenes” is used to describe genes that extend lifespan when their function is abrogated or lost (e.g., the *C. elegans* IGF-1 receptor gene, *daf-2*). “Longevity genes” are typically those that extend lifespan when their function is enhanced (e.g., yeast *SIR2*, *C. elegans* *daf-16*), although often these terms are often used interchangeably, albeit incorrectly.

3.2. Hormesis in budding yeast

The budding yeast *Saccharomyces cerevisiae* has provided the first proof of the Hormesis Hypothesis of CR for any species. Yeast “replicative” lifespan is defined as the number of divisions an individual yeast cell undergoes before dying (Bitterman et al., 2003). One attractive feature of *S. cerevisiae*, as opposed to many other budding yeasts, is that the progenitor cell is easily distinguished from its descendants because cell division is asymmetric: a newly formed “daughter” cell is almost always smaller than the “mother” cell that gave rise to it. Yeast mother cells divide about 20 times before dying and undergo characteristic structural and metabolic changes as they age (Bitterman et al., 2003).

To understand the role of hormesis in yeast lifespan, it is necessary to be familiar with Sir2, a conserved longevity factor involved in the CR response. Only a brief introduction to Sir2 will be given here because recent and comprehensive reviews are available (Hekimi and Guarente, 2003; Lamming et al., 2004; North and Verdin, 2004).

In 1994, Lenny Guarente’s laboratory undertook a genetic screen for mutations that increased yeast replicative lifespan. This led to the identification of a hypermorphic allele of the transcriptional silencing gene *SIR4* (Kennedy et al., 1995). This semi-dominant allele relocalized the *SIR4* protein from yeast telomeres (and mating-type loci) to the ribosomal DNA (rDNA) locus (Kennedy et al., 1997). Around the same time, it was recognized that a major cause of yeast aging stems from the inherent instability of the repetitive rDNA locus (Sinclair and Guarente, 1997). When replication forks stall at the rDNA and a DNA break occurs, a circular molecule of rDNA called an ERC can excise from the locus. This molecule then replicates and stays within the mother cell, thereby accumulating exponentially to toxic levels in mother cells (Sinclair and Guarente, 1997). About 1000 copies per cell are present in the final divisions of the mother, which is more DNA than the yeast genome. Presumably, ERCs kill cells by titrating essential transcription and replication factors (Falcon and Aris, 2003; Sinclair and Guarente, 1997). Based on the ERC hypothesis, and an earlier finding that *SIR2* mutants have highly unstable rDNA (Gottlieb and Esposito, 1989), additional copies of *SIR2* were expected to suppress ERCs and hence lengthen lifespan—and they did (Kaeberlein et al., 1999).

The name “Sir2” stands for “silent information regulator” because with enzyme catalyzes the formation of silent heterochromatin. Additional copies of the *SIR2* gene extend replicative lifespan by about 30% by suppressing rDNA recombination and ERC formation (Kaeberlein et al., 1999). Importantly for this review, Sir2 activity is boosted by CR (Bitterman et al., 2002; Lin et al., 2000). There is at least one alternative pathway of CR independent of *SIR2*, but its identity is not known (Kaeberlein et al., 2004). One possibility is that the alternative pathway involves a *SIR2* homolog (i.e., *HST1-4*) and experiments are underway in our laboratory to test this hypothesis.

Sir2 homologs can be found in a wide array of organisms, from bacteria to humans. In *C. elegans* and *Drosophila*, increased dosage of a Sir2 homolog extends lifespan (Rogina and Helfand, 2004; Tissenbaum and Guarente, 2001). Importantly, loss-of-function mutations in *Drosophila* dSir2 prevent lifespan extension by CR and there is no additive effect of CR and Sir2 overexpression, indicating that dSir2 and CR work via common mechanisms (Rogina and Helfand, 2004).

In mammals, seven Sir2 homologs have been identified: SIRT1–7 (Frye, 1999, 2000). Although it is not yet known whether SIRT1 extends mouse lifespan, there are an increasing number of papers demonstrating that SIRT1 regulates cell survival during adverse conditions. The proteins/pathways that mammalian SIRT1 regulates now include p53 (Motta et al., 2004; Vaziri et al., 2001), Bax-mediated apoptosis (Cohen et al., 2004b), neuronal survival (Araki et al., 2004), the FOXO family of transcription factors (Brunet et al., 2004; Giannakou and Partridge, 2004; Motta et al., 2004; van der Horst et al., 2004), and NF- κ B-mediated cell death (Yeung et al., 2004).

Consistent with the Hormesis Hypothesis, a variety of low-intensity stresses extend yeast lifespan including mild heat, increased salt, low amino acids or low glucose, the yeast equivalent of CR (Anderson et al., 2003; Bitterman et al., 2003; Jiang et al., 2000; Lin et al., 2000). These lifespan-extensions are facilitated by a single gene, *PNC1*, which is induced by every treatment known to extend yeast lifespan (Anderson et al., 2003; Gallo et al., 2004) (Fig. 3). Additional copies of *PNC1* mimic the effects of CR and extend lifespan ~50%. Thus, *PNC1* is a single “master regulatory” gene that unites a variety of treatments that extend lifespan: exactly what the Hormesis Hypothesis predicts. *PNC1* encodes a nicotinamidase that extends yeast lifespan by depleting the cell of nicotinamide (NAM), a physiological inhibitor of Sir2. This system of longevity regulation in yeast explains how multiple, disparate stimuli can lead to the same longevity response and how species can rapidly evolve longevities to suit a new environment (Anderson et al., 2003).

While these findings are intriguing, a big question remains: is there a *PNC1*-like “master regulator” of longevity in mammals? The functional equivalent of *PNC1* in mammals is known as PBEF (a.k.a. NAMPT or Visfatin). Pre-B-cell colony enhancing factor (PBEF) was originally described as a cytokine that increased the number of pre-B-cell colonies above what could be coaxed by stem cell factor and IL-7 (Samal et al., 1994). Recent work has shown that PBEF is also a NAM phosphorybosyltransferase, which catalyzes the first step in the conversion of nicotinamide to NAD⁺ as part of the NAD⁺ salvage pathway (Revollo et al., 2004; Rongvaux et al., 2002).

Consistent with it being a functional equivalent of *PNC1*, PBEF increases SIRT1 activity when overexpressed (Revollo et al., 2004), protects neutrophils against apoptosis (Jia et al., 2004), and unpublished work from our laboratory indicates

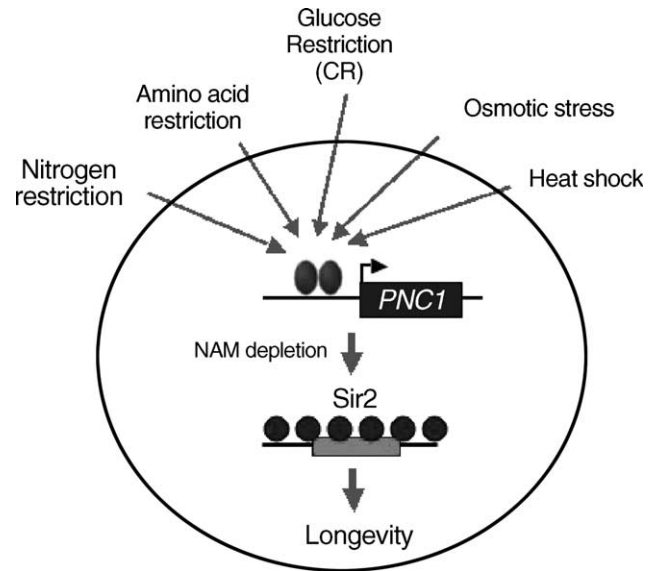


Fig. 3. *S. cerevisiae* lifespan extension by CR is due to hormesis. Replicative lifespan in yeast is extended by glucose restriction and a variety of mild stresses such as heat and salt stress. The Sir2 enzyme, a member of the “sirtuin” family of class III HDACs, is a nicotinamide (NAM)-sensitive deacetylase that extends yeast lifespan by deacetylating histones and stabilizing repetitive DNA. *PNC1* encodes an enzyme that depletes NAM, thereby activating Sir2. *PNC1* can be viewed as a “master regulator of longevity” that serves to translate CR and environmental stress signals into Sir2 activation and longevity (Anderson et al., 2003). Having centralized control of longevity is adaptive because it allows new lifespan strategies to evolve rapidly in response to a changing environment. In mammals, the equivalent enzyme is PBEF (a.k.a. NAMPT or visfatin). PBEF is found in the nucleus where it regulates SIRT1 (Revollo et al., 2004) and is secreted by adipocytes and binds to the insulin receptor at a site different than insulin (Fukuhara et al., 2004). Based on findings in yeast, we hypothesize that PBEF/NAMPT/visfatin is a critical regulator of stress responses, cell survival, and longevity in mammals.

that PBEF is activated by nutrient deprivation and other stressors, recapitulating the *PNC1* story in yeast. Whether the effect of PBEF on SIRT1 activity is primarily due to increased NAD⁺ production/levels, NAM depletion, or a combination of the two, is not yet known (Revollo et al., 2004).

Interestingly, the characterization of visfatin, an adipokine that binds the insulin receptor and stimulates glucose uptake from peripheral tissues, recently led to its identification as PBEF (Fukuhara et al., 2004). The authors of this paper and the accompanying perspective in *Science* did not recognize the connection between PBEF and the NAD⁺ salvage pathway (Fukuhara et al., 2004; Hug and Lodish, 2004), but it could be a crucial link between NAD⁺ biosynthesis, glucose regulation, and lifespan. By analogy to *PNC1* in yeast, we hypothesize that PBEF/NAMPT/visfatin is a regulator of stress resistance, energy metabolism, and possibly longevity in mammals. In the nucleus and cytoplasm, its role would be to regulate the sirtuin family of enzymes and possibly the stress-responsive factor, PARP and recycle nicotinamide, while in serum, it would send a signal to other tissues in the organism that it is time to increase cell defenses and divert energy to them.

3.3. Hormesis in worms and flies

In simple metazoans such as worms and flies, there is ample evidence that the effect of CR is due to hormesis. First, there is a strong correlation between longevity of various strains of worms and flies and their resistance to various types of stress, including desiccation, heat stress, acetone, ethanol, and paraquat (Arking et al., 1991; da Cunha et al., 1995; Harshman et al., 1999; Houthoofd et al., 2003; Mockett et al., 2001; Wang et al., 2004). Second, exposure of organisms to agents or conditions that cause mild biological stress increases lifespan, such as ionizing irradiation, heat, and ROS induced by hyperbaric oxygen treatment (Butov et al., 2001; Cypser and Johnson, 2002, 2003; Johnson and Hartman, 1988; Lithgow et al., 1994; Michalski et al., 2001; Yashin et al., 2001). In worms, many of these stressors have been shown to act through the insulin/IGF-1 pathway by boosting the activity of a conserved forkhead transcription factor, daf-16 (Cypser and Johnson, 2003), which functions to increase the transcription of cell defense genes such as SOD genes and HSP70 (Lee et al., 2003; Murphy et al., 2003). Consistent with this, overexpression of daf-16 target genes, such as HSF-1 (a heat shock transcription factor), HSP16 (a chaperone), SOD-1 (Cu/Zn-dependent superoxide dismutase), or SOD-3 (Mn-dependent), extends worm lifespan (Walker and Lithgow, 2003). Third, exposure of simple animals to mild stress provides cross-resistance to other, seemingly unrelated, stressors such as low doses of paraquat, aldehydes, irradiation, heat shock, crowding, and hypergravity (Braeckman et al., 2001b; Hercus et al., 2003; Lints et al., 1993; Minois et al., 1999; Sorensen and Loeschcke, 2001). Taken together, these observations strongly argue that CR does not simply change metabolism or decrease ROS output, but rather it induces a defense program that provides resistance to a wide array of stresses and protects against the causes of aging. It is worth noting, however, that the relationship of the insulin/IGF-1 pathway to CR is still unclear. Worms and flies with an attenuated insulin/IGF-1 pathway can still respond to CR (Braeckman et al., 2001a; Clancy et al., 2002) indicating either that CR works via different mechanisms to insulin/IGF-1 or that there are redundancies in the CR pathway.

3.4. Evidence of hormesis in mammals

Like simpler eukaryotes, CR increases the resistance of mammals to stress, including sudden increases in temperature and toxins (Duffy et al., 2001; Heydari et al., 1993; Masoro, 1998). Experiments at the cellular and molecular levels also strongly support the Hormesis Hypothesis. For example, fibroblasts from long-lived Snell dwarf mice are relatively resistant to stress and toxins (Murakami et al., 2003) and cells cultured in the presence of serum from CR rats are resistant to stress and pro-apoptotic signals (Cohen et al., 2004b; de Cabo et al., 2003). Subjecting fibroblasts to

repeated heat shock also produces similar effects to those seen *in vivo*, including maintenance of the stress protein profile, reduction in the accumulation of damaged proteins, stimulation of proteolysis, and increased resistance to stressors such as ethanol, hydrogen peroxide, and UV (Rattan, 2004b).

It has recently been demonstrated that the mammalian SIRT1 protein is induced in the tissues of CR rats (Cohen et al., 2004b). At the cellular level, SIRT1 promotes the resistance of cells to stress-induced death by attenuating p53 and stimulating the Ku70-Bax anti-apoptotic system (Cohen et al., 2004a,b; Motta et al., 2004; Vaziri et al., 2001). SIRT1 also stimulates metabolic changes in cells consistent with CR, including increased fatty acid oxidation in adipocytes (Picard et al., 2004) and increases glucose production from hepatocytes via PGC-1 α and PPAR α (Puigserver and Spiegelman, 2004). It will be interesting to examine whether the overexpression of SIRT1 or its homologs, SIRT2-7 in transgenic mice, results in any effects similar to CR.

Although the Hormesis Hypothesis of CR is not yet embraced by the majority of researchers who study CR, it is the best theory we have to explain the multitude of data from lower organisms to mammals. The contrast between this hypothesis and those that preceded it is stark. If this new theory is right, it means that the effect of CR is to provoke an active, functional response to stress, not simply to passively alter metabolism. It means that the response to CR is an ancient one that evolved to promote the survival of organisms during adversity. Being an ancient adaptive response, it also means that discoveries in simple organisms such as yeast, worms, and flies are likely to be more relevant to mammals than many had previously thought. And the most practical implication is that we probably do not need to know the proximal causes of aging to develop life-prolonging strategies; we just need to target the longevity regulators themselves, and they will counter the causes of aging, whatever they may be.

3.5. Implications of the Hormesis Hypothesis: small molecule CR mimetics

In addition to its ability to explain many observations in the field, the Hormesis Hypothesis makes an interesting prediction. If there are innate mechanisms that promote health and survival during stress, it should be possible to design small molecules that can activate them.

Until recently, the search for potential CR mimetic molecules focused solely on those that could modulate energy metabolism. Two prominent examples include 2-deoxyglucose (2DG) and metformin (Dhahbi et al., 2004; Fulgencio et al., 2001; Ingram et al., 2004; Spindler et al., 2003; Wan et al., 2003, 2004; Weindruch et al., 2001) (Fig. 4). 2DG is a synthetic glucose analog that inhibits the glycolytic enzyme phosphohexose isomerase. 2DG injected into rodents suppresses tumor growth, decreases insulin and

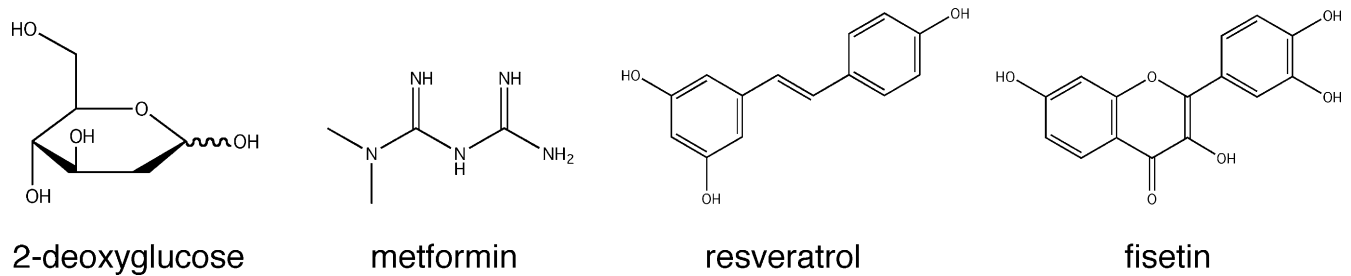


Fig. 4. Known CR mimetics. 2-Deoxyglucose (2DG) is a synthetic glucose analog that inhibits the glycolytic enzyme phosphohexose isomerase. Metformin is a biguanide that originates from the French lilac (*Galega officinalis*) and is used to treat type II diabetes. Although the actual mode of action of metformin is uncertain, recent findings indicate that it acts by activating AMP-activated protein kinase (AMPK) in liver cells, leading to increased fatty acid oxidation and glucose uptake by cells and decreased lipogenesis and hepatic glucose production. Resveratrol is derived from grapes and Asian medicinal herbs, and is in Phase I/II clinical trials for treating HSV-1 infections and colon cancer. Fisetin, derived from Fustet shrubs, is a structurally similar polyphenol. These two molecules extend the lifespan of yeast, worms, and flies 15–70%, apparently by binding to and stimulating the activity of Sir2 deacetylases, thereby altering glucose and fat metabolism, and provoking a variety of cellular defenses and survival pathways.

body temperature, and increases glucocorticoids, all of which parallel CR (Ingram et al., 2004). Unfortunately, chronic administration of 2DG enlarges the heart and increases the chance of congestive heart failure, making it unlikely to extend lifespan of the animals in this ongoing study (Ingram et al., 2004).

Metformin, buformin, and phenformin are members of the biguanide class of drugs that originate from the French lilac (*Galega officinalis*) (Fig. 4). Metformin is currently used to treat type II diabetes. Interestingly, administration of these molecules results in changes that closely parallel the metabolic and gene expression patterns of CR animals (Anisimov et al., 2003; Dilman and Anisimov, 1980). Metformin is thought to work, at least in large part, by stimulating AMP-dependent kinase (AMPK), a cellular energy sensor that modulates appetite, glucose, and insulin metabolism (Carling, 2004). AMPK slows hepatic glucose output by down-regulating expression of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase. In skeletal muscle, it boosts the efficiency of insulin-stimulated glucose uptake by increasing expression of GLUT-4.

Phenformin has been shown to significantly inhibit the incidence of mammary adenocarcinomas in mice by four-fold, to delay the age-related decline in reproductive function, and extend mean and maximum lifespan by 21 and 26%, respectively (Anisimov et al., 2003; Dilman and Anisimov, 1980). Questions arise about long-term safety of these compounds in humans, because biguanides in general increase the risk of lactic acidosis, hence the removal of phenformin from the market. There is ongoing research to find less toxic replacements, which might be suitable as CR mimetics in humans (McCarty, 2004). Taken together, these findings indicate that mimicking CR in mammals by altering glucose/insulin metabolism might be possible, although one cannot rule out the possibility that interfering with metabolic pathways in a normal adult on a normal diet will never be completely safe.

There is, however, a new class of CR mimetic that holds significant promise as a way to prevent disease and extend

maximum lifespan in mammals, without negative side effects. These molecules do not directly target metabolic enzymes but instead boost the activity of the longevity regulators themselves. This class of molecule is thus far limited to those that target the Sir2 family of longevity-promoting enzymes (a.k.a. the “sirtuins”), but no doubt others will be discovered.

Since the discovery that sirtuins are regulators of cell survival and longevity in model organisms, there has been a great deal of interest in finding small molecules that can alter the activity of the enzyme family. Nicotinamide, a product of the Sir2/SIRT1 enzymatic reaction, is an effective inhibitor of this class of enzyme (Anderson et al., 2003; Bitterman et al., 2002) and several synthetic inhibitors have been identified including splitomicin (Bedalov et al., 2001) and sirtuinol (Grozing et al., 2001).

Although sirtuin inhibitors may prove to be useful in treating some diseases, sirtuin activating compounds (STACs) could be the key to extending lifespan in higher organisms. Recently, 18 small molecules from plants were identified that can increase human SIRT1 activity in vitro and in vivo, including resveratrol, butein, and piceatannol (Howitz et al., 2003) (Fig. 4). The compound with the greatest stimulatory activity was resveratrol, a polyphenol that is found in numerous plant species including grapes, peanuts, and some Asian medicinal herbs. Relatively low concentrations of these polyphenols extend lifespan of yeast, *C. elegans* and *Drosophila*, in a Sir2-dependent manner, apparently by mimicking CR (Wood et al., 2004). They are the first molecules to extend the lifespan of multiple, diverse species.

Although it will be years before we know whether resveratrol can extend lifespan in rodents, it does have remarkable effects on mammalian cells in vitro and in vivo consistent with it mimicking CR. It protects cells from a variety of stresses including H₂O₂- and 4-hydroxynonenal-induced oxidative damage (Araki et al., 2004; Kutuk et al., 2004), gamma radiation (Howitz et al., 2003), and Bax-mediated apoptosis (Cohen et al., 2004b). Moreover, a variety of cellular phenotypes have been linked to

resveratrol's ability to stimulate SIRT1 in vivo, including the suppression of NF- κ B inflammatory pathways (Yeung et al., 2004), protection of primary neurons from H₂O₂ (Araki et al., 2004), and the modulation of fatty acid metabolism via PPAR γ (Picard et al., 2004) and glucose metabolism via PGC-1 α (Picard et al., 2004; Puigserver and Spiegelman, 2004).

Even more provocative are observations that resveratrol is effective against numerous, disparate diseases including esophageal, breast, and liver cancers (Bhat and Pezzuto, 2002; Jang et al., 1997), oral herpes (Docherty et al., 1999, 2004), chronic obstructive pulmonary disease (COPD) (Culpitt et al., 2003), and hyperlipidemia (Miura et al., 2003). Resveratrol has also shown efficacy in treating ischemic events (Kiziltepe et al., 2004) possibly via its effects on SIRT1 activity (Alcendor et al., 2004). Two other STACs, quercetin and butein, also show efficacy against age-related diseases (Knekt et al., 2002; Lim et al., 2001). Of course, it is too early to say if resveratrol or the other STACs improve health by triggering a CR-like hormetic effect. It is, however, interesting to consider that these are the type of early findings one might expect of a *bonafide* CR mimetic.

3.6. Sirtuin activating compounds: molecular mimics or xenohormesis?

How can we explain the fact that small molecules produced by plants extend the lifespan of numerous species and are effective against a variety of diseases in rodents? It cannot simply be a coincidence. It is unlikely to be due to the anti-oxidant activity of STACs because oxidative stress does not limit yeast lifespan (Lin et al., 2002), and anti-oxidants typically do not extend lifespan of metazoans (Bauer et al., 2004). There are a variety of plausible explanations, including the possibility that resveratrol mimics the action of an endogenous activator in yeast and animals. Indeed, resveratrol and like polyphenols resemble the basic mammalian glucocorticoid structure and at high doses can be estrogenic.

But there is another intriguing possibility. Resveratrol and other STACs might be plant stress-signaling molecules that coordinate sirtuin-mediated defenses in plants (Howitz et al., 2003). Plants possess multiple Sir2 family members (Frye, 2000; Pandey et al., 2002) and many of the polyphenols that activate the sirtuins, such as resveratrol and quercetin, are known to be synthesized during times of stress such as during infection, starvation, and dehydration. This idea contrasts with the mainstream view of these molecules as anti-oxidants or plant antibiotics (Stojanovic et al., 2001). But why would plant STACs activate sirtuins from yeast and humans? Could it be conservation of the past billion years? This seems highly unlikely.

An intriguing yet controversial explanation is that animals and fungi have retained an ability to be activated by certain plant stress molecules because they provide a useful advance warning of a deteriorating environment or

food supply (Howitz et al., 2003) (Fig. 3). This interspecies communication of stress signals has been termed “xenohormesis” by Howitz and Sinclair. The hypothesis makes a number of predictions. First, we should find a bounty of medicinal molecules in stressed plants. Second, the molecules should be relatively non-toxic since we evolved along with them. Third, the molecules should interact with a variety of enzymes involved in regulating stress responses and longevity, not only the sirtuins.

This last point is important. Although we have discussed the idea of xenohormesis in the context of sirtuin activation, there is no necessity that plant stress-induced compounds should act only on this target. Rather, the Xenohormesis Hypothesis predicts that STACs will act on numerous targets, each working to improve survival of the organism. Indeed, resveratrol is known to modulate numerous targets that are predicted to increase health and/or longevity (Pervaiz, 2003). Examples include the inhibition of cyclooxygenases (Jang et al., 1997), PI 3 kinase (Pozo-Guisado et al., 2004; She et al., 2003), ribonucleotide reductase (Fontecave et al., 1998), and cyclooxygenase 2 (COX2) (Subbaramaiah et al., 1998, 1999). Perhaps, one day we will use xenohormetic molecules widely in medicine to activate our bodies' innate defenses against disease, thereby providing the remarkable health benefits currently reserved for CR animals.

References

- Alcendor, R.R., Kirshenbaum, L.A., Imai, S., Vatner, S.F., Sadoshima, J., 2004. Silent information regulator 2 α , a longevity factor and class III histone deacetylase, is an essential endogenous apoptosis inhibitor in cardiac myocytes. *Circ. Res.* 95, 971–980.
- Anderson, R.M., Bitterman, K.J., Wood, J.G., Medvedik, O., Sinclair, D.A., 2003. Nicotinamide and Pnc1 govern lifespan extension by calorie restriction in *S. cerevisiae*. *Nature* 423, 181–185.
- Ando, K., Higami, Y., Tsuchiya, T., Kanematsu, T., Shimokawa, I., 2002. Impact of aging and life-long calorie restriction on expression of apoptosis-related genes in male F344 rat liver. *Microsc. Res. Tech.* 59, 293–300.
- Anisimov, V.N., Semenchenko, A.V., Yashin, A.I., 2003. Insulin and longevity: antidiabetic biguanides as geroprotectors. *Biogerontology* 4, 297–307.
- Antebi, A., 2004. Tipping the balance toward longevity. *Dev. Cell* 6, 315–316.
- Araki, T., Sasaki, Y., Milbrandt, J., 2004. Increased nuclear NAD biosynthesis and SIRT1 activation prevent axonal degeneration. *Science* 305, 1010–1013.
- Arking, R., Buck, S., Berrios, A., Dwyer, S., Baker 3rd, G.T., 1991. Elevated paraquat resistance can be used as a bioassay for longevity in a genetically based long-lived strain of *Drosophila*. *Dev. Genet.* 12, 362–370.
- Austad, S.N., 2001. Does caloric restriction in the laboratory simply prevent overfeeding and return house mice to their natural level of food intake? *Sci. Aging Knowledge Environ.* 6, 3.
- Barja, G., 2004. Aging in vertebrates, and the effect of caloric restriction: a mitochondrial free radical production–DNA damage mechanism? *Biol. Rev. Camb. Philos. Soc.* 79, 235–251.
- Bartke, A., Coschigano, K., Kopchick, J., Chandrashekar, V., Mattison, J., Kinney, B., Hauck, S., 2001a. Genes that prolong life: relationships of

- growth hormone and growth to aging and life span. *J. Gerontol. A Biol. Sci. Med. Sci.* 56, B340–B349.
- Bartke, A., Wright, J.C., Mattison, J.A., Ingram, D.K., Miller, R.A., Roth, G.S., 2001b. Extending the lifespan of long-lived mice. *Nature* 414, 412.
- Barzilai, N., Gabrieli, I., 2001. The role of fat depletion in the biological benefits of caloric restriction. *J. Nutr.* 131, 903S–906S.
- Barzilai, N., Gupta, G., 1999. Revisiting the role of fat mass in the life extension induced by caloric restriction. *J. Gerontol. A Biol. Sci. Med. Sci.* 54, B89–B96, discussion B97–B98.
- Bauer, J.H., Goupil, S., Garber, G.B., Helfand, S.L., 2004. An accelerated assay for the identification of lifespan-extending interventions in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U.S.A.* 101, 12980–12985.
- Bedalov, A., Gatabont, T., Irvine, W.P., Gottschling, D.E., Simon, J.A., 2001. Identification of a small molecule inhibitor of Sir2p. *Proc. Natl. Acad. Sci. U.S.A.* 98, 15113–15118.
- Berg, B.N., Simms, H.S., 1960. Nutrition and longevity in the rat. II. Longevity and onset of disease with different levels of food intake. *J. Nutr.* 71, 255–263.
- Bertrand, H.A., Lynd, F.T., Masoro, E.J., Yu, B.P., 1980. Changes in adipose mass and cellularity through the adult life of rats fed ad libitum or a life-prolonging restricted diet. *J. Gerontol.* 35, 827–835.
- Bhat, K.P., Pezzuto, J.M., 2002. Cancer chemopreventive activity of resveratrol. *Ann. N. Y. Acad. Sci.* 957, 210–229.
- Bitterman, K.J., Anderson, R.M., Cohen, H.Y., Latorre-Esteves, M., Sinclair, D.A., 2002. Inhibition of silencing and accelerated aging by nicotinamide, a putative negative regulator of yeast sir2 and human SIRT1. *J. Biol. Chem.* 277, 45099–45107.
- Bitterman, K.J., Medvedik, O., Sinclair, D.A., 2003. Longevity regulation in *Saccharomyces cerevisiae*: linking metabolism, genome stability, and heterochromatin. *Microbiol. Mol. Biol. Rev.* 67, 376–399 (table of contents).
- Blüher, M., Kahn, B.B., Kahn, C.R., 2003. Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science* 299, 572–574.
- Braeckman, B.P., Houthoofd, K., Vanfleteren, J.R., 2001a. Insulin-like signaling, metabolism, stress resistance and aging in *Caenorhabditis elegans*. *Mech. Ageing Dev.* 122, 673–693.
- Braeckman, B.P., Houthoofd, K., Vanfleteren, J.R., 2001b. Insulin-like signaling, metabolism, stress resistance and aging in *Caenorhabditis elegans*. *Mech. Ageing Dev.* 122, 673–693.
- Braeckman, B.P., Houthoofd, K., Vanfleteren, J.R., 2002. Assessing metabolic activity in aging *Caenorhabditis elegans*: concepts and controversies. *Ageing Cell* 1, 82–88, discussion 102–103.
- Brunet, A., Sweeney, L.B., Sturgill, J.F., Chua, K.F., Greer, P.L., Lin, Y., Tran, H., Ross, S.E., Mostoslavsky, R., Cohen, H.Y., Hu, L.S., Cheng, H.L., Jedrychowski, M.P., Gygi, S.P., Sinclair, D.A., Alt, F.W., Greenberg, M.E., 2004. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. *Science* 303 (5666), 2011–2015.
- Butov, A., Johnson, T., Cypser, J., Sannikov, I., Volkov, M., Sehl, M., Yashin, A., 2001. Hormesis and debilitation effects in stress experiments using the nematode worm *Caenorhabditis elegans*: the model of balance between cell damage and HSP levels. *Exp. Gerontol.* 37, 57–66.
- Calabrese, E.J., 2004. Hormesis: from marginalization to mainstream: a case for hormesis as the default dose–response model in risk assessment. *Toxicol. Appl. Pharmacol.* 197, 125–136.
- Calabrese, E.J., McCarthy, M.E., Kenyon, E., 1987. The occurrence of chemically induced hormesis. *Health Phys.* 52, 531–541.
- Calingasan, N.Y., Gibson, G.E., 2000. Dietary restriction attenuates the neuronal loss, induction of heme oxygenase-1 and blood–brain barrier breakdown induced by impaired oxidative metabolism. *Brain Res.* 885, 62–69.
- Cao, S.X., Dhahbi, J.M., Mote, P.L., Spindler, S.R., 2001. Genomic profiling of short- and long-term caloric restriction effects in the liver of aging mice. *Proc. Natl. Acad. Sci. U.S.A.* 98, 10630–10635.
- Carling, D., 2004. The AMP-activated protein kinase cascade—a unifying system for energy control. *Trends Biochem. Sci.* 29, 18–24.
- Cartee, G.D., Kietzke, E.W., Briggs-Tung, C., 1994. Adaptation of muscle glucose transport with caloric restriction in adult, middle-aged, and old rats. *Am. J. Physiol.* 266, R1443–R1447.
- Cassano, P., Lezza, A.M., Leeuwenburgh, C., Cantatore, P., Gadaleta, M.N., 2004. Measurement of the 4,834-bp mitochondrial DNA deletion level in aging rat liver and brain subjected or not to caloric restriction diet. *Ann. N. Y. Acad. Sci.* 1019, 269–273.
- Charlesworth, B., 2000. Fisher, Medawar, Hamilton and the evolution of aging. *Genetics* 156, 927–931.
- Clancy, D.J., Gems, D., Hafen, E., Leevers, S.J., Partridge, L., 2002. Dietary restriction in long-lived dwarf flies. *Science* 296, 319.
- Cohen, H.Y., Lavu, S., Bitterman, K.J., Hekking, B., Imahiyerobo, T.A., Miller, C., Frye, R., Ploegh, H., Kessler, B.M., Sinclair, D.A., 2004a. Acetylation of the C terminus of Ku70 by CBP and PCAF controls Bax-mediated apoptosis. *Mol. Cell* 13, 627–638.
- Cohen, H.Y., Miller, C., Bitterman, K.J., Wall, N.R., Hekking, B., Kessler, B., Howitz, K.T., Gorospe, M., de Cabo, R., Sinclair, D.A., 2004b. Caloric restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. *Science* 305, 390–392.
- Culpitt, S.V., Rogers, D.F., Fenwick, P.S., Shah, P., De Matos, C., Russell, R.E., Barnes, P.J., Donnelly, L.E., 2003. Inhibition by red wine extract, resveratrol, of cytokine release by alveolar macrophages in COPD. *Thorax* 58, 942–946.
- Cutler, R.G., 1982. Longevity is determined by specific genes: testing the hypothesis. In: Adelman, R.C., Roth, G.S. (Eds.), *Testing the Theories of Aging*. CRC Press, Inc., Boca Raton, FL, pp. 24–114.
- Cypser, J.R., Johnson, T.E., 2002. Multiple stressors in *Caenorhabditis elegans* induce stress hormesis and extended longevity. *J. Gerontol. A Biol. Sci. Med. Sci.* 57, B109–B114.
- Cypser, J.R., Johnson, T.E., 2003. Hormesis in *Caenorhabditis elegans* dauer-defective mutants. *Biogerontology* 4, 203–214.
- D’Mello, N.P., Childress, A.M., Franklin, D.S., Kale, S.P., Pinswasdi, C., Jazwinski, S.M., 1994. Cloning and characterization of LAG1, a longevity-assurance gene in yeast. *J. Biol. Chem.* 269, 15451–15459.
- da Cunha, G.L., da Cruz, I.B., Fiorino, P., de Oliveira, A.K., 1995. Paraquat resistance and starvation conditions in the selection of longevity extremes in *Drosophila melanogaster* populations previously selected for long and short developmental period. *Dev. Genet.* 17, 352–361.
- de Cabo, R., Furer-Galban, S., Anson, R.M., Gilman, C., Gorospe, M., Lane, M.A., 2003. An in vitro model of caloric restriction. *Exp. Gerontol.* 38, 631–639.
- Dean, D.J., Brozinick Jr., J.T., Cushman, S.W., Cartee, G.D., 1998a. Calorie restriction increases cell surface GLUT-4 in insulin-stimulated skeletal muscle. *Am. J. Physiol.* 275, E957–E964.
- Dean, D.J., Gazdag, A.C., Wetter, T.J., Cartee, G.D., 1998b. Comparison of the effects of 20 days and 15 months of calorie restriction on male Fischer 344 rats. *Ageing (Milano)* 10, 303–307.
- Dehmelt, H., 2004. Re-adaptation hypothesis: explaining health benefits of caloric restriction. *Med. Hypotheses* 62, 620–624.
- Del Roso, A., Vittorini, S., Cavallini, G., Donati, A., Gori, Z., Masini, M., Pollera, M., Bergamini, E., 2003. Ageing-related changes in the in vivo function of rat liver macroautophagy and proteolysis. *Exp. Gerontol.* 38, 519–527.
- Dhahbi, J.M., Kim, H.J., Mote, P.L., Beaver, R.J., Spindler, S.R., 2004. Temporal linkage between the phenotypic and genomic responses to caloric restriction. *Proc. Natl. Acad. Sci. U.S.A.* 101, 5524–5529.
- Dilman, V.M., Anisimov, V.N., 1980. Effect of treatment with phenformin, diphenylhydantoin or L-dopa on life span and tumour incidence in C3H/Sn mice. *Gerontology* 26, 241–246.
- Docherty, J.J., Fu, M.M., Stiffler, B.S., Limperos, R.J., Pokabla, C.M., DeLucia, A.L., 1999. Resveratrol inhibition of herpes simplex virus replication. *Antivir. Res.* 43, 145–155.
- Docherty, J.J., Smith, J.S., Fu, M.M., Stoner, T., Booth, T., 2004. Effect of topically applied resveratrol on cutaneous herpes simplex virus infections in hairless mice. *Antivir. Res.* 61, 19–26.
- Droge, W., 2003. Oxidative stress and aging. *Adv. Exp. Med. Biol.* 543, 191–200.

- Duffy, P.H., Seng, J.E., Lewis, S.M., Mayhugh, M.A., Aidoo, A., Hattan, D.G., Casciano, D.A., Feuers, R.J., 2001. The effects of different levels of dietary restriction on aging and survival in the Sprague–Dawley rat: implications for chronic studies. *Aging (Milano)* 13, 263–272.
- Dufour, E., Larsson, N.G., 2004. Understanding aging: revealing order out of chaos. *Biochim. Biophys. Acta* 1658, 122–132.
- Falcon, A.A., Aris, J.P., 2003. Plasmid accumulation reduces life span in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 278, 41607–41617.
- Fontecave, M., Lepoivre, M., Elleingand, E., Gerez, C., Guittet, O., 1998. Resveratrol, a remarkable inhibitor of ribonucleotide reductase. *FEBS Lett.* 421, 277–279.
- Friedman, D.B., Johnson, T.E., 1988. A mutation in the age-1 gene in *Caenorhabditis elegans* lengthens life and reduces hermaphrodite fertility. *Genetics* 118, 75–86.
- Frye, R.A., 1999. Characterization of five human cDNAs with homology to the yeast SIR2 gene: Sir2-like proteins (sirtuins) metabolize NAD and may have protein ADP-ribosyltransferase activity. *Biochem. Biophys. Res. Commun.* 260, 273–279.
- Frye, R.A., 2000. Phylogenetic classification of prokaryotic and eukaryotic Sir2-like proteins. *Biochem. Biophys. Res. Commun.* 273, 793–798.
- Fukuhara, A., Matsuda, M., Nishizawa, M., Segawa, K., Tanaka, M., Kishimoto, K., Matsuki, Y., Murakami, M., Ichisaka, T., Murakami, H., Watanabe, E., Takagi, T., Akiyoshi, M., Ohtsubo, T., Kihara, S., Yamashita, S., Makishima, M., Funahashi, T., Yamanaka, S., Hiramatsu, R., Matsuzawa, Y., Shimomura, I., 2004. Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. *Science* 307 (5708), 426–433.
- Fulgencio, J.P., Kohl, C., Girard, J., Pegorier, J.P., 2001. Effect of metformin on fatty acid and glucose metabolism in freshly isolated hepatocytes and on specific gene expression in cultured hepatocytes. *Biochem. Pharmacol.* 62, 439–446.
- Gallo, C.M., Smith Jr., D.L., Smith, J.S., 2004. Nicotinamide clearance by Pnc1 directly regulates Sir2-mediated silencing and longevity. *Mol. Cell Biol.* 24, 1301–1312.
- Giannakou, M.E., Partridge, L., 2004. The interaction between FOXO and SIRT1: tipping the balance towards survival. *Trends Cell Biol.* 14, 408–412.
- Gong, X., Shang, F., Obin, M., Palmer, H., Scrofano, M.M., Jahngen-Hodge, J., Smith, D.E., Taylor, A., 1997. Antioxidant enzyme activities in lens, liver and kidney of calorie restricted Emory mice. *Mech. Ageing Dev.* 99, 181–192.
- Gottlieb, S., Esposito, R.E., 1989. A new role for a yeast transcriptional silencer gene, SIR2, in regulation of recombination in ribosomal DNA. *Cell* 56, 771–776.
- Groinger, C.C.E., Blackwell, H.E., Moazed, D., Schreiber, S.L., 2001. Identification of a class of small molecule inhibitors of the sirtuin family of NAD-dependent deacetylases by phenotypic screening. *J. Biol. Chem.* 276, 38837–38843.
- Guo, Z.M., Yang, H., Hamilton, M.L., VanRemmen, H., Richardson, A., 2001. Effects of age and food restriction on oxidative DNA damage and antioxidant enzyme activities in the mouse aorta. *Mech. Ageing Dev.* 122, 1771–1786.
- Gupta, G., Cases, J.A., She, L., Ma, X.H., Yang, X.M., Hu, M., Wu, J., Rossetti, L., Barzilai, N., 2000. Ability of insulin to modulate hepatic glucose production in aging rats is impaired by fat accumulation. *Am. J. Physiol. Endocrinol. Metab.* 278, E985–E991.
- Harrison, D.E., Archer, J.R., Astle, C.M., 1984. Effects of food restriction on aging: separation of food intake and adiposity. *Proc. Natl. Acad. Sci. U.S.A.* 81, 1835–1838.
- Harshman, L.G., Moore, K.M., Sty, M.A., Magwire, M.M., 1999. Stress resistance and longevity in selected lines of *Drosophila melanogaster*. *Neurobiol. Aging* 20, 521–529.
- Hatano, E., Tanaka, A., Kanazawa, A., Tsuyuki, S., Tsunekawa, S., Iwata, S., Takahashi, R., Chance, B., Yamaoka, Y., 2004. Inhibition of tumor necrosis factor-induced apoptosis in transgenic mouse liver expressing creatine kinase. *Liver Int.* 24, 384–393.
- Hayflick, L., 1994. *How and Why We Age*. Ballantine, New York.
- Hekimi, S., Guarente, L., 2003. Genetics and the specificity of the aging process. *Science* 299, 1351–1354.
- Hercus, M.J., Loeschcke, V., Rattan, S.I., 2003. Lifespan extension of *Drosophila melanogaster* through hormesis by repeated mild heat stress. *Biogerontology* 4, 149–156.
- Heydari, A.R., Wu, B., Takahashi, R., Strong, R., Richardson, A., 1993. Expression of heat shock protein 70 is altered by age and diet at the level of transcription. *Mol. Cell Biol.* 13, 2909–2918.
- Hiona, A., Leeuwenburgh, C., 2004. Effects of age and caloric restriction on brain neuronal cell death/survival. *Ann. N. Y. Acad. Sci.* 1019, 96–105.
- Holliday, R., 1989. Food, reproduction and longevity: is the extended lifespan of calorie-restricted animals an evolutionary adaptation? *Bioessays* 10, 125–127.
- Houthoofd, K., Braeckman, B.P., Johnson, T.E., Vanfleteren, J.R., 2003. Life extension via dietary restriction is independent of the Ins/IGF-1 signalling pathway in *Caenorhabditis elegans*. *Exp. Gerontol.* 38, 947–954.
- Houthoofd, K., Braeckman, B.P., Lenaerts, I., Brys, K., De Vreese, A., Van Eygen, S., Vanfleteren, J.R., 2002. Axenic growth up-regulates mass-specific metabolic rate, stress resistance, and extends life span in *Caenorhabditis elegans*. *Exp. Gerontol.* 37, 1371–1378.
- Howitz, K.T., Bitterman, K.J., Cohen, H.Y., Lamming, D.W., Lavu, S., Wood, J.G., Zipkin, R.E., Chung, P., Kiselewski, A., Zhang, L.L., Scherer, B., Sinclair, D.A., 2003. Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature* 425, 191–196.
- Howitz, K.T., Sinclair, D.A., 2005. Dietary restriction, hormesis, and small molecule mimetics. In: Masoro, E.J., Austad, S.N. (Eds.), *Handbook of the Biology of Aging*. 6th edn. Academic Press, London.
- Hug, C., Lodish, H.F., 2004. Visfatin: a new adipokine. *Science* 307 (5708), 366–367.
- Hulbert, A.J., Clancy, D.J., Mair, W., Braeckman, B.P., Gems, D., Partridge, L., 2004. Metabolic rate is not reduced by dietary-restriction or by lowered insulin/IGF-1 signalling and is not correlated with individual lifespan in *Drosophila melanogaster*. *Exp. Gerontol.* 39, 1137–1143.
- Ikeyama, S., Wang, X.T., Li, J., Podlitsky, A., Martindale, J.L., Kokkonen, G., van Huizen, R., Gorospe, M., Holbrook, N.J., 2003. Expression of the pro-apoptotic gene gadd153/chop is elevated in liver with aging and sensitizes cells to oxidant injury. *J. Biol. Chem.* 278, 16726–16731.
- Ingram, D.K., Anson, R.M., de Cabo, R., Mamczarz, J., Zhu, M., Mattison, J., Lane, M.A., Roth, G.S., 2004. Development of calorie restriction mimetics as a pro-longevity strategy. *Ann. N. Y. Acad. Sci.* 1019, 412–423.
- Izmaylov, D.M., Obukhova, L.K., 1999. Geroprotector effectiveness of melatonin: investigation of lifespan of *Drosophila melanogaster*. *Mech. Ageing Dev.* 106, 233–240.
- James, S.J., Muskhelishvili, L., Gaylor, D.W., Turturro, A., Hart, R., 1998. Upregulation of apoptosis with dietary restriction: implications for carcinogenesis and aging. *Environ. Health Perspect.* 106 (Suppl. 1), 307–312.
- Jang, M., Cai, L., Udeani, G.O., Slowing, K.V., Thomas, C.F., Beecher, C.W., Fong, H.H., Farnsworth, N.R., Kinghorn, A.D., Mehta, R.G., Moon, R.C., Pezzuto, J.M., 1997. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science* 275, 218–220.
- Jia, S.H., Li, Y., Parodo, J., Kapus, A., Fan, L., Rotstein, O.D., Marshall, J.C., 2004. Pre-B cell colony-enhancing factor inhibits neutrophil apoptosis in experimental inflammation and clinical sepsis. *J. Clin. Invest.* 113, 1318–1327.
- Jiang, J.C., Jaruga, E., Repnevskaya, M.V., Jazwinski, S.M., 2000. An intervention resembling caloric restriction prolongs life span and retards aging in yeast. *FASEB J.* 14, 2135–2137.
- Johnson, T.E., Hartman, P.S., 1988. Radiation effects on life span in *Caenorhabditis elegans*. *J. Gerontol.* 43, B137–B141.
- Kaerberlein, M., Kirkland, K.T., Fields, S., Kennedy, B.K., 2004. Sir2-independent life span extension by calorie restriction in yeast. *PLoS Biol.* 2, E296.

- Kaeberlein, M., McVey, M., Guarente, L., 1999. The SIR2/3/4 complex and SIR2 alone promote longevity in *Saccharomyces cerevisiae* by two different mechanisms. *Genes Dev.* 13, 2570–2580.
- Kalant, N., Stewart, J., Kaplan, R., 1988. Effect of diet restriction on glucose metabolism and insulin responsiveness in aging rats. *Mech. Ageing Dev.* 46, 89–104.
- Kennedy, B.K., Austriaco Jr., N.R., Zhang, J., Guarente, L., 1995. Mutation in the silencing gene SIR4 can delay aging in *S. cerevisiae*. *Cell* 80, 485–496.
- Kennedy, B.K., Gotta, M., Sinclair, D.A., Mills, K., McNabb, D.S., Murthy, M., Pak, S.M., Laroche, T., Gasser, S.M., Guarente, L., 1997. Redistribution of silencing proteins from telomeres to the nucleolus is associated with extension of life span in *S. cerevisiae*. *Cell* 89, 381–391.
- Kenyon, C., Chang, J., Gensch, E., Rudner, A., Tabtiang, R., 1993. A *C. elegans* mutant that lives twice as long as wild type. *Nature* 366, 461–464.
- Khavinson, V.K., Izmaylov, D.M., Obukhova, L.K., Malinin, V.V., 2000. Effect of epitalon on the lifespan increase in *Drosophila melanogaster*. *Mech. Ageing Dev.* 120, 141–149.
- Kirkwood, T.B., Holliday, R., 1979. The evolution of ageing and longevity. *Proc. R. Soc. Lond. B Biol. Sci.* 205, 531–546.
- Kiziltepe, U., Turan, N.N., Han, U., Ulus, A.T., Akar, F., 2004. Resveratrol, a red wine polyphenol, protects spinal cord from ischemia-reperfusion injury. *J. Vasc. Surg.* 40, 138–145.
- Knekt, P., Kumpulainen, J., Jarvinen, R., Rissanen, H., Heliövaara, M., Reunanen, A., Hakulinen, T., Aromaa, A., 2002. Flavonoid intake and risk of chronic diseases. *Am. J. Clin. Nutr.* 76, 560–568.
- Koubova, J., Guarente, L., 2003. How does calorie restriction work? *Genes Dev.* 17, 313–321.
- Krystal, B.S., Yu, B.P., 1994. Aging and its modulation by dietary restriction. In: Yu, B.P. (Ed.), *Modulation of Aging Processes by Dietary Restriction*. CRC Press, London, pp. 1–36.
- Kutuk, O., Adli, M., Poli, G., Basaga, H., 2004. Resveratrol protects against 4-HNE induced oxidative stress and apoptosis in Swiss 3T3 fibroblasts. *Biofactors* 20, 1–10.
- Lamming, D.W., Wood, J.G., Sinclair, D.A., 2004. Small molecules that regulate lifespan: evidence for xenohormesis. *Mol. Microbiol.* 53, 1003–1009.
- Lee, J.H., Jung, K.J., Kim, J.W., Kim, H.J., Yu, B.P., Chung, H.Y., 2004. Suppression of apoptosis by calorie restriction in aged kidney. *Exp. Gerontol.* 39, 1361–1368.
- Lee, S.S., Kennedy, S., Tolonen, A.C., Ruvkun, G., 2003. DAF-16 target genes that control *C. elegans* life-span and metabolism. *Science* 300, 644–647.
- Lewis, S.E., Goldspink, D.F., Phillips, J.G., Merry, B.J., Holehan, A.M., 1985. The effects of aging and chronic dietary restriction on whole body growth and protein turnover in the rat. *Exp. Gerontol.* 20, 253–263.
- Lim, S.S., Jung, S.H., Ji, J., Shin, K.H., Keum, S.R., 2001. Synthesis of flavonoids and their effects on aldose reductase and sorbitol accumulation in streptozotocin-induced diabetic rat tissues. *J. Pharm. Pharmacol.* 53, 653–668.
- Lin, S.J., Defossez, P.A., Guarente, L., 2000. Requirement of NAD and SIR2 for life-span extension by calorie restriction in *Saccharomyces cerevisiae*. *Science* 289, 2126–2128.
- Lin, S.J., Kaeberlein, M., Andalis, A.A., Sturtz, L.A., Defossez, P.A., Culotta, V.C., Fink, G.R., Guarente, L., 2002. Calorie restriction extends *Saccharomyces cerevisiae* lifespan by increasing respiration. *Nature* 418, 344–348.
- Lindsay, D.G., 1999. Diet and ageing: the possible relation to reactive oxygen species. *J. Nutr. Health Aging* 3, 84–91.
- Lints, F.A., Bullens, P., Le Bourg, E., 1993. Hypergravity and aging in *Drosophila melanogaster*: 7. New longevity data. *Exp. Gerontol.* 28, 611–615.
- Lithgow, G.J., 2001. Hormesis—a new hope for ageing studies or a poor second to genetics? *Hum. Exp. Toxicol.* 20, 301–303, discussion 319–320.
- Lithgow, G.J., White, T.M., Hinerfeld, D.A., Johnson, T.E., 1994. Thermotolerance of a long-lived mutant of *Caenorhabditis elegans*. *J. Gerontol.* 49, B270–B276.
- Luhtala, T.A., Roecker, E.B., Pugh, T., Feuers, R.J., Weindruch, R., 1994. Dietary restriction attenuates age-related increases in rat skeletal muscle antioxidant enzyme activities. *J. Gerontol.* 49, B231–B238.
- Masoro, E.J., 1992. Potential role of the modulation of fuel use in the antiaging action of dietary restriction. *Ann. N. Y. Acad. Sci.* 663, 403–411.
- Masoro, E.J., 1995. Antiaging action of caloric restriction: endocrine and metabolic aspects. *Obes. Res.* 3 (Suppl. 2), 241s–247s.
- Masoro, E.J., 1996. Possible mechanisms underlying the antiaging actions of caloric restriction. *Toxicol. Pathol.* 24, 738–741.
- Masoro, E.J., 1998. Influence of caloric intake on aging and on the response to stressors. *J. Toxicol. Environ. Health B Crit. Rev.* 1, 243–257.
- Masoro, E.J., 2000. Caloric restriction and aging: an update. *Exp. Gerontol.* 35, 299–305.
- Masoro, E.J., Austad, S.N., 1996. The evolution of the antiaging action of dietary restriction: a hypothesis. *J. Gerontol. A Biol. Sci. Med. Sci.* 51, B387–B391.
- Masoro, E.J., Yu, B.P., Bertrand, H.A., 1982. Action of food restriction in delaying the aging process. *Proc. Natl. Acad. Sci. U.S.A.* 79, 4239–4241.
- Masternak, M.M., Al-Regaiey, K., Bonkowski, M.S., Panici, J., Sun, L., Wang, J., Przybylski, G.K., Bartke, A., 2004. Divergent effects of caloric restriction on gene expression in normal and long-lived mice. *J. Gerontol. A Biol. Sci. Med. Sci.* 59, 784–788.
- Mattson, M.P., Chan, S.L., Duan, W., 2002a. Modification of brain aging and neurodegenerative disorders by genes, diet, and behavior. *Physiol. Rev.* 82, 637–672.
- Mattson, M.P., Duan, W., Chan, S.L., Cheng, A., Haughey, N., Gary, D.S., Guo, Z., Lee, J., Furukawa, K., 2002b. Neuroprotective and neurorestorative signal transduction mechanisms in brain aging: modification by genes, diet and behavior. *Neurobiol. Aging* 23, 695–705.
- McCarter, R., Masoro, E.J., Yu, B.P., 1985. Does food restriction retard aging by reducing the metabolic rate? *Am. J. Physiol.* 248, E488–E490.
- McCarty, M.F., 2004. Chronic activation of AMP-activated kinase as a strategy for slowing aging. *Med. Hypotheses* 63, 334–339.
- McCay, C.M., 1935. Cellulose in the diet of mice and rats. *J. Nutr.* 435–447.
- McCay, C.M., Crowell, M.F., Maynard, L.A., 1935. The effect of retarded growth upon the length of lifespan and upon the ultimate body size. *J. Nutr.* 10, 63–79.
- McCay, C.M., Maynard, L.A., Sperlberg, G., Barnes, L.L., 1975. Retarded growth, life span, ultimate body size and age changes in the albino rat after feeding diets restricted in calories. *Nutr. Rev.* 33, 241–243.
- Medawar, P.B., 1946. Old age and natural death. *Mod. Q.* 1, 30–56.
- Merry, B.J., 2002. Molecular mechanisms linking calorie restriction and longevity. *Int. J. Biochem. Cell Biol.* 34, 1340–1354.
- Merry, B.J., 2004. Oxidative stress and mitochondrial function with aging—the effects of calorie restriction. *Ageing Cell* 3, 7–12.
- Michalski, A.I., Johnson, T.E., Cypser, J.R., Yashin, A.I., 2001. Heating stress patterns in *Caenorhabditis elegans* longevity and survivorship. *Biogerontology* 2, 35–44.
- Migliaccio, E., Giorgio, M., Mele, S., Pellicci, G., Reboldi, P., Pandolfi, P.P., Lanfrancone, L., Pellicci, P.G., 1999. The p66shc adaptor protein controls oxidative stress response and life span in mammals. *Nature* 402, 309–313.
- Miller, R.A., Chang, Y., Galecki, A.T., Al-Regaiey, K., Kopchick, J.J., Bartke, A., 2002. Gene expression patterns in calorically restricted mice: partial overlap with long-lived mutant mice. *Mol. Endocrinol.* 16, 2657–2666.
- Minois, N., Guinaudy, M.J., Payre, F., Le Bourg, E., 1999. HSP70 induction may explain the long-lasting resistance to heat of *Drosophila melanogaster* having lived in hypergravity. *Mech. Ageing Dev.* 109, 65–77.
- Miura, D., Miura, Y., Yagasaki, K., 2003. Hypolipidemic action of dietary resveratrol, a phytoalexin in grapes and red wine, in hepatoma-bearing rats. *Life Sci.* 73, 1393–1400.

- Miwa, S., Riyahi, K., Partridge, L., Brand, M.D., 2004. Lack of correlation between mitochondrial reactive oxygen species production and life span in *Drosophila*. *Ann. N. Y. Acad. Sci.* 1019, 388–391.
- Mobbs, C.V., Bray, G.A., Atkinson, R.L., Bartke, A., Finch, C.E., Maratos-Flier, E., Crawley, J.N., Nelson, J.F., 2001. Neuroendocrine and pharmacological manipulations to assess how caloric restriction increases life span. *J. Gerontol. A Biol. Sci. Med. Sci.* 56 (Spec. No. 1), 34–44.
- Mockett, R.J., Orr, W.C., Rahmandar, J.J., Sohal, B.H., Sohal, R.S., 2001. Antioxidant status and stress resistance in long- and short-lived lines of *Drosophila melanogaster*. *Exp. Gerontol.* 36, 441–463.
- Monti, B., Contestabile, A., 2003. Selective alteration of DNA fragmentation and caspase activity in the spinal cord of aged rats and effect of dietary restriction. *Brain Res.* 992, 137–141.
- Motta, M.C., Divecha, N., Lemieux, M., Kamel, C., Chen, D., Gu, W., Bultsma, Y., McBurney, M., Guarente, L., 2004. Mammalian SIRT1 represses forkhead transcription factors. *Cell* 116, 551–563.
- Mukherjee, P., Abate, L.E., Seyfried, T.N., 2004. Antiangiogenic and proapoptotic effects of dietary restriction on experimental mouse and human brain tumors. *Clin. Cancer Res.* 10, 5622–5629.
- Murakami, S., Salmon, A., Miller, R.A., 2003. Multiplex stress resistance in cells from long-lived dwarf mice. *FASEB J.* 17, 1565–1566.
- Murphy, C.T., McCarroll, S.A., Bargmann, C.I., Fraser, A., Kamath, R.S., Ahringer, J., Li, H., Kenyon, C., 2003. Genes that act downstream of DAF-16 to influence the lifespan of *Caenorhabditis elegans*. *Nature* 424, 277–283.
- Nelson, J.F., Karelus, K., Bergman, M.D., Felicio, L.S., 1995. Neuroendocrine involvement in aging: evidence from studies of reproductive aging and caloric restriction. *Neurobiol. Aging* 16, 837–843, discussion 855–856.
- North, B.J., Verdin, E., 2004. Sirtuins: Sir2-related NAD-dependent protein deacetylases. *Genome Biol.* 5, 224.
- Pandey, R., Muller, A., Napoli, C.A., Selinger, D.A., Pikaard, C.S., Richards, E.J., Bender, J., Mount, D.W., Jorgensen, R.A., 2002. Analysis of histone acetyltransferase and histone deacetylase families of *Arabidopsis thaliana* suggests functional diversification of chromatin modification among multicellular eukaryotes. *Nucleic Acids Res.* 30, 5036–5055.
- Parkes, T.L., Elia, A.J., Dickinson, D., Hilliker, A.J., Phillips, J.P., Boulianne, G.L., 1998. Extension of *Drosophila* lifespan by overexpression of human SOD1 in motorneurons. *Nat. Genet.* 19, 171–174.
- Pearl, R., 1928. *The Rate of Living*. Alfred Knopf, New York, pp. 183–185.
- Pervaiz, S., 2003. Resveratrol: from grapevines to mammalian biology. *FASEB J.* 17, 1975–1985.
- Picard, F., Kurtev, M., Chung, N., Topark-Ngarm, A., Senawong, T., Machado De Oliveira, R., Leid, M., McBurney, M.W., Guarente, L., 2004. Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR- γ . *Nature* 429, 771–776.
- Pozo-Guisado, E., Lorenzo-Benayas, M.J., Fernandez-Salguero, P.M., 2004. Resveratrol modulates the phosphoinositide 3-kinase pathway through an estrogen receptor α -dependent mechanism: relevance in cell proliferation. *Int. J. Cancer* 109, 167–173.
- Pugh, T.D., Klopp, R.G., Weindruch, R., 1999. Controlling caloric consumption: protocols for rodents and rhesus monkeys. *Neurobiol. Aging* 20, 157–165.
- Puigserver, P., Spiegelman, B., 2004. Personal communication.
- Rattan, S.I., 1998. Repeated mild heat shock delays ageing in cultured human skin fibroblasts. *Biochem. Mol. Biol. Int.* 45, 753–759.
- Rattan, S.I., 2004a. Aging, anti-aging, and hormesis. *Mech. Ageing Dev.* 125, 285–289.
- Rattan, S.I., 2004b. Hormetic mechanisms of anti-aging and rejuvenating effects of repeated mild heat stress on human fibroblasts in vitro. *Rejuvenation Res.* 7, 40–48.
- Reveillaud, I., Kongpachith, A., Park, R., Fleming, J.E., 1992. Stress resistance of *Drosophila* transgenic for bovine CuZn superoxide dismutase. *Free Radic. Res. Commun.* 17, 73–85.
- Revollo, J.R., Grimm, A.A., Imai, S., 2004. The NAD biosynthesis pathway mediated by nicotinamide phosphoribosyltransferase regulates Sir2 activity in mammalian cells. *J. Biol. Chem.* 279, 50754–50763.
- Rogina, B., Helfand, S.L., 2004. Sir2 mediates longevity in the fly through a pathway related to calorie restriction. *Proc. Natl. Acad. Sci. U.S.A.* 101, 15998–16003.
- Rojas, C., Cadenas, S., Perez-Campo, R., Lopez-Torres, M., Pamplona, R., Prat, J., Barja, G., 1993. Relationship between lipid peroxidation, fatty acid composition, and ascorbic acid in the liver during carbohydrate and caloric restriction in mice. *Arch. Biochem. Biophys.* 306, 59–64.
- Rongvaux, A., Shea, R.J., Mulks, M.H., Gigot, D., Urbain, J., Leo, O., Andris, F., 2002. Pre-B-cell colony-enhancing factor, whose expression is up-regulated in activated lymphocytes, is a nicotinamide phosphoribosyltransferase, a cytosolic enzyme involved in NAD biosynthesis. *Eur. J. Immunol.* 32, 3225–3234.
- Rose, M.R., Vu, L.N., Park, S.U., Graves Jr., J.L., 1992. Selection on stress resistance increases longevity in *Drosophila melanogaster*. *Exp. Gerontol.* 27, 241–250.
- Rubner, M., 1908. *Das problem der Lebensdauer und seine Beziehungen zum Wachstum unter Ernahrung*. Oldenburg, Munich, pp. 150–204.
- Samal, B., Sun, Y., Stearns, G., Xie, C., Suggs, S., McNiece, I., 1994. Cloning and characterization of the cDNA encoding a novel human pre-B-cell colony-enhancing factor. *Mol. Cell Biol.* 14, 1431–1437.
- Selman, C., Gredilla, R., Phaneuf, S., Kendaiah, S., Barja, G., Leeuwenburgh, C., 2003. Short-term caloric restriction and regulatory proteins of apoptosis in heart, skeletal muscle and kidney of Fischer 344 rats. *Biogerontology* 4, 141–147.
- Shaddock, J.G., Feuers, R.J., Chou, M.W., Swenson, D.H., Casciano, D.A., 1995. Genotoxicity of tacrine in primary hepatocytes isolated from B6C3F1 mice and aged ad libitum and calorie restricted Fischer 344 rats. *Mutat. Res.* 344, 79–88.
- She, Q.B., Ma, W.Y., Wang, M., Kaji, A., Ho, C.T., Dong, Z., 2003. Inhibition of cell transformation by resveratrol and its derivatives: differential effects and mechanisms involved. *Oncogene* 22, 2143–2150.
- Shelke, R.R., Leeuwenburgh, C., 2003. Lifelong caloric restriction increases expression of apoptosis repressor with a caspase recruitment domain (ARC) in the brain. *FASEB J.* 17, 494–496.
- Sinclair, D.A., 1999. Yeast aging research: recent advances and medical relevance. *Cell Mol. Life Sci.* 56, 807–816.
- Sinclair, D.A., Guarente, L., 1997. Extrachromosomal rDNA circles—a cause of aging in yeast. *Cell* 91, 1033–1042.
- Smith, J.M., 1958. Prolongation of the life of *Drosophila subobscura* by a brief exposure of adults to a high temperature. *Nature* 181, 496–497.
- Sorensen, J.G., Loeschcke, V., 2001. Larval crowding in *Drosophila melanogaster* induces Hsp70 expression, and leads to increased adult longevity and adult thermal stress resistance. *J. Insect Physiol.* 47, 1301–1307.
- Southam, C.M., Ehrlich, J., 1943. Effects of extract of western red-cedar heartwood on certain wood decaying fungi in culture. *Phytopathology* 33, 517–524.
- Speakman, J.R., Talbot, D.A., Selman, C., Snart, S., McLaren, J.S., Redman, P., Krol, E., Jackson, D.M., Johnson, M.S., Brand, M.D., 2004. Uncoupled and surviving: individual mice with high metabolism have greater mitochondrial uncoupling and live longer. *Aging Cell* 3, 87–95.
- Speakman, J.R., van Acker, A., Harper, E.J., 2003. Age-related changes in the metabolism and body composition of three dog breeds and their relationship to life expectancy. *Aging Cell* 2, 265–275.
- Spencer, C.C., Howell, C.E., Wright, A.R., Promislow, D.E., 2003. Testing an ‘aging gene’ in long-lived *Drosophila* strains: increased longevity depends on sex and genetic background. *Aging Cell* 2, 123–130.
- Spindler, S.R., Dhahbi, J.M., Mote, P.L., Kim, H.J., Tshuchiya, T., 2003. Rapid identification of candidate CR mimetics using microarrays. *Biogerontology* 4, 89.
- Spindler, S.R., Grizzle, J.M., Walford, R.L., Mote, P.L., 1991. Aging and restriction of dietary calories increases insulin receptor mRNA, and aging increases glucocorticoid receptor mRNA in the liver of female C3B10RF1 mice. *J. Gerontol.* 46, B233–B237.

- Stadtman, E.R., 1995. Role of oxidized amino acids in protein breakdown and stability. *Methods Enzymol.* 258, 379–393.
- Stojanovic, S., Sprinz, H., Brede, O., 2001. Efficiency and mechanism of the antioxidant action of trans-resveratrol and its analogues in the radical liposome oxidation. *Arch. Biochem. Biophys.* 391, 79–89.
- Strehler, B.L., 1967. Environmental factors in aging and mortality. *Environ. Res.* 1, 46–88.
- Stuart, J.A., Karahalil, B., Hogue, B.A., Souza-Pinto, N.C., Bohr, V.A., 2004. Mitochondrial and nuclear DNA base excision repair are affected differently by caloric restriction. *FASEB J.* 18, 595–597.
- Subbaramaiah, K., Chung, W.J., Michaluart, P., Telang, N., Tanabe, T., Inoue, H., Jang, M., Pezzuto, J.M., Dannenberg, A.J., 1998. Resveratrol inhibits cyclooxygenase-2 transcription and activity in phorbol ester-treated human mammary epithelial cells. *J. Biol. Chem.* 273, 21875–21882.
- Subbaramaiah, K., Michaluart, P., Chung, W.J., Tanabe, T., Telang, N., Dannenberg, A.J., 1999. Resveratrol inhibits cyclooxygenase-2 transcription in human mammary epithelial cells. *Ann. N. Y. Acad. Sci.* 889, 214–223.
- Tanaka, K., Higami, Y., Tsuchiya, T., Shiokawa, D., Tanuma, S., Ayabe, H., Shimokawa, I., 2004. Aging increases DNase gamma, an apoptosis-related endonuclease, in rat liver nuclei: effect of dietary restriction. *Exp. Gerontol.* 39, 195–202.
- Tatar, M., Bartke, A., Antebi, A., 2003. The endocrine regulation of aging by insulin-like signals. *Science* 299, 1346–1351.
- Tavernarakis, N., Driscoll, M., 2002. Caloric restriction and lifespan: a role for protein turnover? *Mech. Ageing Dev.* 123, 215–229.
- Tissenbaum, H.A., Guarente, L., 2001. Increased dosage of a sir-2 gene extends lifespan in *Caenorhabditis elegans*. *Nature* 410, 227–230.
- Trifunovic, A., Wredenberg, A., Falkenberg, M., Spelbrink, J.N., Rovio, A.T., Bruder, C.E., Bohlooly, Y.M., Gidlof, S., Oldfors, A., Wibom, R., Tornell, J., Jacobs, H.T., Larsson, N.G., 2004. Premature ageing in mice expressing defective mitochondrial DNA polymerase. *Nature* 429, 417–423.
- Tsuchiya, T., Dhahbi, J.M., Cui, X., Mote, P.L., Bartke, A., Spindler, S.R., 2004. Additive regulation of hepatic gene expression by dwarfism and caloric restriction. *Physiol. Genomics* 17, 307–315.
- Turturro, A., Hass, B., Hart, R.W., 1998. Hormesis—implications for risk assessment caloric intake (body weight) as an exemplar. *Hum. Exp. Toxicol.* 17, 454–459.
- Turturro, A., Hass, B.S., Hart, R.W., 2000. Does caloric restriction induce hormesis? *Hum. Exp. Toxicol.* 19, 320–329.
- van der Horst, A., Tertoolen, L.G., de Vries-Smits, L.M., Frye, R.A., Medema, R.H., Burgering, B.M., 2004. FOXO4 is acetylated upon peroxide stress and deacetylated by the longevity protein hSir2(SIRT1). *J. Biol. Chem.* 279, 28873–28879.
- Van Remmen, H., Ikeno, Y., Hamilton, M., Pahlavani, M., Wolf, N., Thorpe, S.R., Alderson, N.L., Baynes, J.W., Epstein, C.J., Huang, T.T., Nelson, J., Strong, R., Richardson, A., 2003. Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging. *Physiol. Genomics* 16, 29–37.
- Vaziri, H., Dessain, S.K., Eaton, E.N., Imai, S.I., Frye, R.A., Pandita, T.K., Guarente, L., Weinberg, R.A., 2001. hSIR2(SIRT1) functions as an NAD-dependent p53 deacetylase. *Cell* 107, 149–159.
- Wachsmann, J.T., 1996. The beneficial effects of dietary restriction: reduced oxidative damage and enhanced apoptosis. *Mutat. Res.* 350, 25–34.
- Walker, G.A., Lithgow, G.J., 2003. Lifespan extension in *C. elegans* by a molecular chaperone dependent upon insulin-like signals. *Ageing Cell* 2, 131–139.
- Wan, R., Camandola, S., Mattson, M.P., 2003. Intermittent fasting and dietary supplementation with 2-deoxy-D-glucose improve functional and metabolic cardiovascular risk factors in rats. *FASEB J.* 17, 1133–1134.
- Wan, R., Camandola, S., Mattson, M.P., 2004. Dietary supplementation with 2-deoxy-D-glucose improves cardiovascular and neuroendocrine stress adaptation in rats. *Am. J. Physiol. Heart Circ. Physiol.* 287, H1186–H1193.
- Wang, H.D., Kazemi-Esfarjani, P., Benzer, S., 2004. Multiple-stress analysis for isolation of *Drosophila* longevity genes. *Proc. Natl. Acad. Sci. U.S.A.* 101, 12610–12615.
- Weindruch, R., Kayo, T., Lee, C.K., Prolla, T.A., 2001. Microarray profiling of gene expression in aging and its alteration by caloric restriction in mice. *J. Nutr.* 131, 918S–923S.
- Weindruch, R., Walford, R.L., 1988. *The Retardation of Aging and Disease by Dietary Restriction*. Charles C. Thomas, Springfield, IL.
- Westerman, J.M., Parsons, P.A., 1972. Radioresistance and longevity of inbred lines of *Drosophila melanogaster*. *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.* 21, 145–152.
- Williams, G.C., 1957. Pleiotropy, natural selection, and the evolution of senescence. *Evolution* 11, 398–411.
- Wood, J.G., Rogina, B., Lavu, S., Howitz, K., Helfand, S.L., Tatar, M., Sinclair, D., 2004. Sirtuin activators mimic caloric restriction and delay ageing in metazoans. *Nature* 430, 686–689.
- Yashin, A.I., Cypser, J.R., Johnson, T.E., Michalski, A.I., Boyko, S.I., Novoseltsev, V.N., 2001. Ageing and survival after different doses of heat shock: the results of analysis of data from stress experiments with the nematode worm *Caenorhabditis elegans*. *Mech. Ageing Dev.* 122, 1477–1495.
- Yeung, F., Hoberg, J.E., Ramsey, C.S., Keller, M.D., Jones, D.R., Frye, R.A., Mayo, M.W., 2004. Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. *EMBO J.* 23, 2369–2380.
- Yu, B.P., Masoro, E.J., McMahan, C.A., 1985. Nutritional influences on aging of Fischer 344 rats: I. Physical, metabolic, and longevity characteristics. *J. Gerontol.* 40, 657–670.
- Yuneva, A.O., Kramarenko, G.G., Vetreshchak, T.V., Gallant, S., Boldyrev, A.A., 2002. Effect of carnosine on *Drosophila melanogaster* lifespan. *Bull. Exp. Biol. Med.* 133, 559–561.
- Zhang, Y., Herman, B., 2002. Ageing and apoptosis. *Mech. Ageing Dev.* 123, 245–260.