Calorie restriction mimetics: an emerging research field

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Summary

When considering all possible aging interventions evaluated to date, it is clear that calorie restriction (CR) remains the most robust. Studies in numerous species have demonstrated that reduction of calories 30–50% below ad libitum levels of a nutritious diet can increase lifespan, reduce the incidence and delay the onset of age-related diseases, improve stress resistance, and decelerate functional decline. A current major focus of this research area is whether this nutritional intervention is relevant to human aging. Evidence emerging from studies in rhesus monkeys suggests that their response to CR parallels that observed in rodents. To assess CR effects in humans, clinical trials have been initiated. However, even if results from these studies could eventually substantiate CR as an effective pro-longevity strategy for humans, the utility of this intervention would be hampered because of the degree and length of restriction required. As an alternative strategy, new research has focused on the development of ‘CR mimetics’. The objective of this strategy is to identify compounds that mimic CR effects by targeting metabolic and stress response pathways affected by CR, but without actually restricting caloric intake. For example, drugs that inhibit glycolysis (2-deoxyglucose), enhance insulin action (metformin), or affect stress signaling pathways (resveratrol), are being assessed as CR mimetics (CRM). Promising results have emerged from initial studies regarding physiological responses which resemble those observed in CR (e.g. reduced body temperature and plasma insulin) as well as protection against neurotoxicity (e.g. enhanced dopamine action and up-regulated neurotrophic factors). Ultimately, lifespan analyses in addition to expanded toxicity studies must be accomplished to fully assess the potential of any CRM. Nonetheless, this strategy clearly offers a very promising and expanding research endeavor.

Key words: aging; cancer; glucose; insulin; metabolism; stress

Introduction

It is intriguing that one of the oldest paradigms in gerontology is providing the newest synthesis of findings regarding mechanisms of aging and suggesting directions for intervention that could prove to be highly fruitful. The formal study of calorie restriction (CR), also known as food restriction or diet restriction, is most often traced to the pioneering nutritional research of Clive McCay and colleagues at Cornell University beginning in the 1930s (McCay & Crowell, 1934; McCay et al., 1935). As reviewed by Kritchesky (1993), however, reports of CR effects on cancer growth appeared much earlier in the work of More-schi in 1909 and Rous in 1914. Results of many subsequent studies in different laboratories have demonstrated that substantial reductions of food intake to 30–60% below ad libitum (AL) intake levels can increase lifespan (median and maximum) in a wide variety of species (Weindruch & Walford, 1988; Yu, 1994; Weindruch & Sohal, 1997; Masoro, 2000). Moreover, in hundreds of rodent studies ranging over several decades, CR has been shown to decrease the incidence and age of onset of many age-related diseases, increase resistance to toxicity and stress, and maintain function at more youthful-like states compared to controls eating at or near AL levels (Weindruch & Walford, 1988; Yu, 1994). Compared to numerous attempts to manipulate the rate of aging in animal models, CR remains the most robust aging intervention studied to date.

Given its robust nature and continued research prominence, the three most compelling questions driving recent CR research are: (i) Is this intervention relevant to human aging? (ii) If so, can mechanisms of CR relevant to human aging be identified? (iii) If so, can interventions be identified that operate through these mechanisms to mimic CR?

Relevance of calorie restriction to humans

Arguments have been presented that the marked increases in lifespan observed in rodents on CR are not likely to be observed in humans on CR because humans have not evolved such mechanisms (Harrison & Archer, 1989; de Grey, 2005; Phelan & Rose, 2005). CR may be more relevant to short-lived species in which a major loss of food supply would be catastrophic, whereas humans have evolved cultural mechanisms for dealing with such events and might not have to rely on such evolutionary adaptation. Using a mathematical model relating longevity to relative
investment in reproductive effort, Phelan & Rose (2005) have estimated that CR should have 10 times greater effects on longevity of rodents than of humans.

Of course, many epidemiological studies have reported that caloric intake is related to the incidence of several chronic diseases, including cardiovascular disease, cancer, diabetes, as well as neurodegenerative disorders (Logrosino et al., 1996; Mayeux et al., 1999; Willet, 2000; Astrup, 2001; Kritchesky, 2001; Hursting et al., 2003). Studies of Okinawan centenarians support the view that a low-calorie diet can increase prospects for good health and longevity in humans (Suzuki et al., 2001). Findings from the small group of volunteers confined to Biosphere 2 confirmed that CR (~30%) could be imposed for 2 years and would produce many of the physiological, hormonal, and morphological effects expected (Walford et al., 2002). A recent study of self-selecting volunteers on CR also noted remarkably low values on risk factors for cardiovascular disease (Fontana et al., 2004). These studies are valuable and clearly suggestive of the relationship between caloric intake and aging but are no substitute for experimental evidence.

Experimental proof that CR can significantly increase lifespan in organisms that live longer than rodents has been limited to one study in dogs (Kealy et al., 2002) and one in cows (Pinney et al., 1972). Studies in nonhuman primates have indicated that many physiological responses in monkeys parallel those observed in rodents on CR (Mattison et al., 2003; Roth et al., 2004). In addition, primate studies relating several of these responses to markers of disease risk, such as blood lipids and hormones, also indicate a reduced incidence of chronic diseases, such as heart disease and diabetes (Roth et al., 2001).

To assess the feasibility and potential of CR intervention in humans, recent clinical studies sponsored by the National Institute on Aging were initiated at three sites – Washington University, Tufts University, and the Pennington Center at Louisiana State University (Heilbronn & Ravussin, 2003). These pilot studies have now been completed and provide valuable information on physiological responses to CR over a short-term (6–12 months) as well as greatly needed information on procedures to obtain and maintain compliance for such regimens. Another study which imposed alternate-day fasting was also completed by investigators at the Pennington Center which showed that such regimens could produce many CR-like effects but was questionable as a long-term diet strategy because of negative reports of hunger (Heilbronn et al., 2005).

Even if science could advance to a point where it was indeed clear that 30–40% CR imposed in humans could reduce morbidity and mortality and maintain function to the same degree as observed in rodents, questions would remain; that is, could we do it? and would we do it? When considering the difficulty that Westerners have in maintaining low-calorie diets for prolonged periods (Burke et al., 1997; Knauper et al., 2005), much less for a lifetime, the prospects for successful application of CR to humans on a broad scale would appear dim due to the severity and length of restriction required. The realization of this challenge has generated interest in developing alternatives to CR, or calorie restriction mimetics, that can provide the pro-longevity benefits of CR without actually having to reduce caloric intake.

**Calorie restriction mimetics**

The field of calorie restriction mimetics (CRM) is still in its infancy but is attracting increasing attention (Lane et al., 2002; Ingram et al., 2004; Everitt et al., 2005; Roth et al., 2005). A recent search yielded 21 citations, the oldest citation dated 2001. Ironically, one of the citations that does not appear on PubMed is the paper by Lane et al. (1998) that first introduced the concept and the first candidate CRM drug.

Unfortunately since that initial report, the concept itself has evolved to a current context of broad connotation. To many researchers, CRM applies to any intervention that can evoke similar effects on aging, health, and lifespan to those of CR. Such interventions can include antioxidants, hormonal replacement, metabolic enhancers, metal chelators, and even exercise (Hadley et al., 2001; Poehlman et al., 2001). In addition, appetite suppressants and even gastric bypass surgery might also qualify as CRM because they reduce caloric intake.

We would argue that the concept of CRM should have a much more focused meaning and application. Specifically, we propose the following features for any candidate CRM: (i) it mimics the metabolic, hormonal, and physiological effects of CR; (ii) it does not significantly reduce long-term food intake; (iii) it activates stress response pathways observed in CR and provides protection against a variety of stressors; and (iv) it produces CR-like effects on longevity, reduction of age-related disease and maintenance of function.

Taking this approach, the major question is what would be the appropriate mechanism/s to target for a CRM? Several mechanisms underlying the pro-longevity effects of CR have been proposed and include the following: (i) reduced oxidative stress and damage (Sohal & Weindruch, 1996); (ii) reduced glycation of macromolecules (Sell et al., 1996); (iii) reduced DNA damage and increased repair (Raffoul et al., 1999); (iv) reduced inflammation and autoimmunity (Chung et al., 2001); (v) increased mitochondrial metabolic efficiency to protect the plasma membrane (de Cabo et al., 2004); (vi) reduced damage to cellular components, such as lysosomes and peroxisomes, that negatively impact autophagic proteolysis (Bergamini et al., 2004); (vii) enhanced maintenance of age-related patterns of gene expression (Weindruch et al., 2001); and (viii) enhanced protection against stress, or hormesis (Masoro, 1998; Rattan, 2001). Certainly it is possible that interventions providing protection against many of the deleterious processes listed above could produce CR-like effects, but would such interventions produce the robust, multisystem effects associated with CR?

**Hormesis**

In a recent, insightful review of CR and CRM, Sinclair (2005) pointed out that hypotheses pertaining to mechanisms of CR have for the most part been based on the view that underlying
mechanisms were passive in their protective modes. In contrast, the hormesis hypothesis of CR posits an active role of stress protection. Specifically, CR imposes a low level of stress on the organism that in turn activates stress responses providing protection against a variety of aging processes. The concept of hormesis is derived from toxicology where the term implies that small doses of a toxin might have long-term beneficial consequences as a means of conditioning the organism toward enhanced stress responses (Masoro & Austad, 1996; Rattan, 2001).

Such a concept would be in tune with an evolutionary perspective provided by the disposable soma theory of aging, which proposes that when faced with low energy availability, an organism must shift its energy investment away from growth and reproductive processes to energy investment in somatic maintenance and repair (Shanley & Kirkwood, 2000). This compelling hypothesis would predict that it is adaptive for the organism under CR to use available energy to enhance its protection against stress (Masoro & Austad, 1996). It is well established that CR induces stress, manifested as higher levels of circulating glucocorticoids (Masoro & Austad, 1996). Evidence of enhanced stress responses in rodents on CR has been produced in numerous paradigms (Masoro & Austad, 1996), for example, increased resistance to a variety of neurotoxins in rats and mice on various regimens of CR (Bruce-Keller et al., 1999; Zhu et al., 1999; Calinagasan & Gibson, 2000; Duan et al., 2001). Based on this perspective, we propose that it would be productive to develop CRM that could target a broadly acting mechanism, such as an enhanced stress protection. The objective should be to trick the organism into a CR state and thereby activate the protective mechanisms that are induced in CR. Moreover, the parallel objective should be to minimize any reduction in actual caloric consumption. This latter objective is driven more by scientific interests than by practical considerations. CRM can best be evaluated when this objective of avoiding actual CR is achieved; otherwise, one is merely evaluating CR.

**Downstream vs. upstream mechanisms**

In his review, Sinclair (2005) agrees strongly with the hormesis hypothesis and emphasizes the importance of identifying the cellular and genetic mechanisms so that CRM could directly target the ‘longevity regulators’. Such efforts have thus focused on downstream, nuclear positioned mechanisms of CR, such as the sirtuin class of genes (Sir2 and SIRT1, primarily) that act as histone deacetylases regulating gene expression. Considerable experimental support for the involvement of this pathway has been provided in many invertebrate studies. For example in Drosophila, mutations that reduce the levels of the histone deacetylases, Rdp3 deacetylase (Rogina et al., 2002) and over-expression of Sir2 (Rogina & Helfand, 2004) increase lifespan. In addition, when CR is imposed in Rdp3 mutants or in mutants that reduce Sir2, then no increase in lifespan is observed.

In addition to downstream targets, we contend that CRM can also be developed for specific upstream targets involved in energy metabolism. Our initial efforts in developing the concept of CRM have targeted energy pathways to mimic the physiological responses of CR and to enhance stress responses. This strategy emerged from the growing evidence that energy sensing pathways appear critical in regulating aging in a number of model systems.

A widely cited example of the evolutionarily conserved and adaptive strategy of CR is the diapause found in the nematode, Caenorhabditis elegans. When placed in low-energy environments, this small roundworm transforms to its dauer state in which development and reproduction are arrested. Within the dauer state, the nematode becomes more stress resistant and can manifest a lifespan exceeding that of its normal adult form (Johnson et al., 2001; Lithgow & Walker, 2002). Careful research has helped to identify several key genes that regulate the conversion to the dauer form, among them age-1, daf-2, daf-16, daf-18, akt-1, akt-2 (Vanfleteren & Braeckman, 1999). This signal transduction pathway is now considered to be homologous to the mammalian insulin/insulin-like growth factor-1 (IGF-1) pathway (Gems & Partridge, 2001; Johnson et al., 2001; Lithgow & Walker, 2002; Tatar et al., 2003). When selected mutations of genes regulating this pathway are produced, for example in daf-2, signaling through this pathway is reduced to a point that the worm does not transform to the dauer form but still exhibits increased stress protection and extended lifespan (Gems & Partridge, 2001; Tatar et al., 2003).

These findings regarding reduced signaling through the insulin/IGF-1 pathways have also been observed in mammalian models with evidence of increased lifespan (Holzenberger et al., 2003). For example, knockout mice for the IGF-1 receptor show increased lifespan and increased insulin resistance to oxidative stress (Masternak et al., 2005). No other phenotypic effects are observed in this mouse as their energy metabolism, nutrient uptake, physical activity, fertility and reproduction do not appear to differ from normal littermates. Similar results of lifespan extension have been reported from studies of transgenic rats (Shimokawa et al., 2002) and mutant dwarf mice (Bartke et al., 2001) in which signaling through growth hormone/IGF-1 pathways has been reduced, but these mutants differ markedly in phenotypic characteristics from their normal controls.

Many investigators interpret findings from these studies of invertebrates and vertebrates as reasons for attempting to identify single genes as the longevity regulators and, thus, the most promising targets for driving the development of CRM (Sinclair, 2005). However, an equally important conclusion to draw from these studies is that manipulations of energy-processing pathways can be made at various upstream and downstream points with similar effects. When considering that downstream targets might be more effective than upstream targets for the production of CRM (Sinclair, 2005), it is important to remember that CR effects are produced by a very upstream manipulation – reducing the amount food available to the organism. Thus, as a screen for identifying candidate CRM, we would argue for a strategy that manipulates systems involved in energy sensing, regulation, and metabolism and then to look for physiological hallmarks of CR and enhanced stress protection in relevant model systems.
**Glycolytic inhibition**

In the first publication directly proposing the development of CRM, Lane et al. (1998) targeted glucose metabolism, specifically to reduce glycolysis without reducing food intake. For this initial study, the compound selected was 2-deoxy-D-glucose (2DG), which inhibits the enzyme phosphohexose isomerase and thus reduces glycolytic processing. Results of previous rodent studies had shown that injections of 2DG could inhibit tumor growth (Gridley et al., 1985), produce torpor (Dark et al., 1994) and increase glucocorticoids (Weidenfeld et al., 1994), all indicative of a CRM. In the initial evaluation of 2DG as a CRM, young male Fischer-344 (F344) rats were fed diets supplemented (by weight) with 0.2%, 0.4%, or 0.6% 2DG, approximating doses of 100–150, 250–300, or 400–450 mg kg\(^{-1}\), respectively. Within the first few weeks, the high dose proved to be toxic, resulting in a number of deaths; thus, this group was then fed the 0.6% diet every other week which appeared to be well tolerated. Toxicity is predicted with a high 2DG dose because of insufficient cellular energy due to an intolerably high degree of glycolysis inhibition. After all, CR works only to a particular degree of restriction, typically 60%.

The 2DG diets produced physiological endpoints indicative of CR. As presented in Figs 1 and 2, plasma insulin and body temperature were reduced in rats on the 0.4% and 0.6% concentration 2DG diets, without significant reduction in plasma glucose. While all doses initially reduced food intake and body weight, the 0.2% and 0.4% groups caught up to controls within a few weeks and by the end of the study at 6 months of age, they did not differ significantly from controls, while the 0.6% group did maintain reduced body weights throughout the experiment. The major findings from this initial study were that a 2DG diet could affect two major biomarkers of CR, specifically to reduce insulin and body temperature, with little or no effects on food intake and body weight. Therefore, 2DG appeared as a promising CRM candidate.

However, why should effects on only two physiological markers evoke such confidence in the potential of this compound? First, we would argue that reduced body temperature and insulin levels are highly indicative of the altered metabolic state that CR produces. Second, the validity of these biomarkers of CR was further confirmed in an analysis of survival data in human males derived from the Baltimore Longitudinal Study of Aging (BLSA). Analysis of the BLSA data revealed that the probability of survival in a healthy sample of men was greater in those with the lowest temperature and plasma insulin levels (Roth et al., 2001).

In addition to the *in vivo* data emerging from the initial studies of 2DG, other *in vitro* studies confirmed the ability of the compound to enhance stress protection. For example, 2DG protected against glutamate excitotoxicity in fetal hippocampal neurons (Lee et al., 1999). Confirmation of up-regulated stress responses observed in CR rats was produced in 2DG treated cultures showing, specifically, increased heat shock protein-70 (HSP-70) and glutamate responsive protein-78 (GRP-78). After 12 weeks of 2DG injections in rats, their cortical synaptosomes exhibited greater protection against iron and amyloid-\(\beta\)-peptide (Guo & Mattson, 2000). In addition, HSP-70 and GRP-78 were significantly elevated compared to control synaptosome preparations (Guo & Mattson, 2000).

Other short-term *in vivo* studies have provided further confirmation that 2DG can enhance stress protection. As examples, in a model of focal ischemia using middle cerebral artery occlusion-reperfusion, 2DG treatment attenuated damage similar to the degree observed in CR (Yu & Mattson, 1999). When mice are injected with the neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine...
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(MPTP), they show motor impairments related to depletion of dopamine resulting from neuronal loss in the substantia nigra; however, 2DG treatments provided greater protection against MPTP and faster behavioral recovery (Duan & Mattson, 1999). Additionally, similar to in vitro results, 2DG treatment again increased HSP-70 and GRP-78 in the DA-rich brain regions of these mice.

Results from a recent long-term study in Sprague-Dawley rats comparing 2DG feeding to CR also reported CRM effects of this compound (Wan et al., 2003). Compared to AL controls, CR (fed every other day) and 2DG-fed groups alike had lower serum glucose and insulin concentrations as well as reduced heart rate and blood pressure but not body temperature. However, food intake and body weight of 2DG-fed rats were similar to controls unlike the markedly reduced body weight of CR rats. In a follow-up study, Wan et al. (2004) demonstrated that 2DG-fed rats also exhibited increased recovery from stress measured as heart rate, blood pressure, and body temperature following restraint and cold water stress.

Other 2DG studies have documented very interesting effects on the brain to indicate additional parallels to CR. For example, in studies of both CR and 2DG, the neurotrophic factor, brain-derived neurotrophic factor (BDNF), has been reported in several studies (Mattson et al., 2003; Mattson, 2005) to be up-regulated in specific regions of the rat brain, although a recent study of hippocampal BDNF in F344 × BN rats did not support this finding (Newton et al., 2005). BDNF is involved in enhancing dopamine neurotransmission and has been shown to be neuroprotective (Hyman et al., 1991). Results of other studies have demonstrated that short-term CR (e.g. 2 weeks) can increase dopamine-related locomotor responses in rats (Fuemayor & Diaz, 1984). Specifically, rats exhibit hyperactivity when challenged with the dopamine agonist, amphetamine, and this response is enhanced in CR rats. In a recent study from our laboratory, it has been shown that rats on CR for 4 months exhibit an enhanced locomotor response to amphetamine, and this response is also observed in rats fed a 0.4% 2DG diet over the same period (Mamczarz et al., 2005). These results are depicted in Fig. 3, which presents locomotor responses first to placement within a novel environment followed by response to a saline injection and then to the amphetamine injection. The increased locomotor response of the 40% CR group relative to controls following amphetamine treatment is readily evident. However, the initial response of the 2DG group is equivalent to that of the CR group although the locomotor response dissipates more quickly in this group compared to CR animals. It was noteworthy in this study that the 2DG-fed rats did not differ in body weight from AL fed controls. This behavioral paradigm might also prove highly useful for screening candidate CRM. Previously it has been noted that CR can attenuate the age-related loss of striatal dopamine D2 receptors (Roth et al., 1984). It would be interesting to determine if long-term 2DG feeding could produce similar results.

Beneficial effects of 2DG injections on tumor growth had been reported previously, but a recent study involving 2DG feeding has shown that a dietary treatment at concentrations of the compound that do not affect food intake and body weight is also highly effective. Specifically, Zhu et al. (2005) reported that 2DG attenuated mammary tumor growth in female Sprague-Dawley rats produced by injection of 1-methyl-1-nitrosourea. Moreover, these investigators used dietary concentrations of 0.02% and 0.03% 2DG after determining that the concentrations used in the Lane et al. (1998) study produced major body weight growth inhibition in these rats. Indeed, they found that 2DG concentrations of 0.06% disrupted normal body weight growth. Even at the low concentrations used, 2DG still reduced serum insulin and raised serum corticosterone levels but had no significant effects on glucose, leptin or IGF-1-values.

It is clear that results have accumulated to suggest that 2DG presents a highly favorable profile as a CRM (Kang & Hwang, 2006).
The next major objective then would be to complete a long-term study to evaluate the effects of the compound on mortality and morbidity. To meet this objective, a study of 2DG feeding (0.25% and 0.4%) was initiated in 6-month-old male F344 rats. After about 2 months on the diet, deaths were noted in the higher dose group, and these accelerated with time. Based on preliminary pathological analysis, the primary cause of death was congestive heart failure manifested as enlarged hearts with marked vacuolation, which had been noted in the initial study in younger rats (Lane et al., 1998). We are currently completing this analysis in addition to assessing whether this cardiac pathology is observed in other rat strains. Notably Zhu et al. (2005) did not report this pathology, but these investigators used much lower doses of 2DG.

Many targets for inhibiting glycolysis exist; therefore, many potential candidates could have potential efficacy as CRM. Targets could include glucose transporters as well as other enzymatic steps, such as hexokinase inhibitors. For example, another compound, iodoacetate acid, which inhibits glyceraldehyde-3-phosphate, has shown potential as a CRM based on in vitro analysis. Pretreatment of fetal hippocampal neurons with this compound provided protection against several stresses, including excessive glutamate, iron, and trophic factor withdrawal and also stimulated an up-regulation of heat shock proteins, HSP70 and HSP90 (Guo et al., 2001).

**IGF-1**

Evidence emerging from studies of dwarf mice has made a strong case for the role of growth hormone and IGF-1 pathways in aging and longevity (Bartke, 2005). However, the phenotypic complexity of these mutants made it difficult to specify exactly which pathways were involved. Moreover, CR was equally effective in lifespan extension in these mice compared to controls. A more recent study using a specific knockout of the IGF-1 receptor reported increased lifespan, and, additionally, CR was ineffective in further lifespan extension (Bartke, 2005). Thus, these results would support a major involvement of IGF-1 in the pro-longevity effects of CR.

Similar effects on lifespan associated with manipulating the insulin/IGF-like signaling (IIS) pathway has also been observed in Drosophila, but the role of IIS in flies does not appear to be as robust as that observed in mice. The chico mutation in the IIS pathway produces dwarf long-lived flies at normal nutrition, and responds with increased lifespan under appropriate conditions of CR, thus leading to the conclusion that CR mechanisms of pro-longevity overlap with those involving IIS (Clancy et al., 2002). Using mutations of the insulin/IGF-1-like (IIl) pathway in nematodes with various regimens of CR, Houthoofd et al. (2003) concluded that the pro-longevity effects of CR were independent of this pathway. In Drosophila, it appears that other signaling pathways, particularly the target of rapamycin (TOR), appear to be critical for lifespan effects. For example, overexpression of TSC1 and TSC2 (tuberous sclerosis complex genes 1 and 2) can inhibit TOR and increase lifespan substantially (Kapahi et al., 2004).

In their review of the literature, Hursting et al. (2003) also argued for the primary role of IGF-1 in the antiaging and anticancer effects of CR. A number of possible candidates for inhibiting the IGF-1 pathway were identified in this review including retinoids (e.g. fenretinide and all-trans retinoic acid), soy isoflavones (e.g. genistein and daidzein), flavonoids (e.g. quercetin and kaempferol), somatostatin analogues (e.g. octreotide, and selective estrogen receptor modulators such as tamoxifen). Other strategies could include small molecule inhibitors of IGF-1 as well as antisense IGF-1. Regarding 2DG in this context, it is important to note in the Zhu et al. (2005) study described previously, there was no effect of the 2DG supplemented diets on plasma IGF-1 levels. Nonetheless, manipulations of IGF-1 remain a viable strategy for developing CRM.
Insulin sensitizers

Compounds that increase sensitivity to insulin offer another area for development of CRM. As described previously, lower circulating insulin is a key biomarker of CR and appears predictive of longevity (Roth et al., 2001). Anisimov (2003) provided an excellent review of possible interventions that affect insulin/IGF-1 signaling pathways with a major emphasis on the biguanides. Dilman (1971) was instrumental in bringing attention to the class of compounds that are derived from the French lilac (Galega officinalis) as potential antidiabetic drugs. Compounds of interest include phenformin, buformin, and metformin, which collectively have been shown to increase glucose utilization and reduce hyperglycemia, free fatty acid utilization, gluconeogenesis, intestinal glucose absorption, serum lipids, insulin, and somatomedin. In a number of rodent studies, phenformin has been shown to increase lifespan and reduce cancer (Anisimov, 2003). Regarding stress protection, phenformin has been reported to increase resistance to glutamate toxicity in primary neuronal cultures (Lee et al., 2002). Clinical use of phenformin in the treatment of type 2 diabetes was attempted, but the drug was removed from the market due to the severe lactic acidosis observed in a number of patients. Metformin is now a widely prescribed medication for treating type 2 diabetes that can be used alone or in combination with sulfonylurea drugs (Kirpichnikov et al., 2002). In addition to improving the metabolic profile of diabetes, increased survival from all-cause mortality has been associated with metformin treatment in both diabetic and cardiovascular disease patients (Scarpello, 2003; Eurich et al., 2005).

In an impressive study in mice evaluating the potential of several candidate CRM using microarray profiling, Dhahbi et al. (2005) reported that the 8 weeks’ treatment with metformin produced a gene expression pattern that was closely aligned with long-term CR. Other candidate CRM were also evaluated in this study including glipizide, rosiglitazone, and soy isoflavone, but the metformin profile was far superior to these compounds in reproducing the gene profile of CR. The authors made a strong case for the utility of this technology in screening candidate CRM.

Sirtuins

Growing interest in the sirtuins as possible downstream mediators of longevity in a number of organisms has stimulated the development of sirtuin-activating compounds, or STACs (Sinclair, 2005). Howitz et al. (2003) described the results of a screen to identify activators of human SIRT1 and reported 18 small molecules, including butein, piceatannol, and resveratrol. Of these molecules, resveratrol has received the most attention. This polyphenol has been shown to increase lifespan in nematodes and fruit flies in a Sir2-dependent manner that appeared to mimic CR (Wood et al., 2004). In vitro studies have also demonstrated its abilities to protect against a wide range of stressors, including oxidative stress, radiation, and ischemia (Sinclair, 2005). In addition, epidemiological studies have indicated that resveratrol consumption can lower the risk of many age-related diseases, including cancer (Sinclair, 2005). Presumably then, resveratrol can meet the criteria presented above as a CRM although these pro-longevity effects have yet to be confirmed in mammalian systems. Nonetheless, the results are certainly promising. In addition, research on a variety of other STACs, with possibly even more robust effects than resveratrol, continue to emerge, including particularly plant polyphenolics (Quideau, 2004; Sinclair, 2005).

PPARs and thiazolidinediones

Other possible candidates as CRM likely exist among the large class of agonists for peroxisome proliferator activated receptors (PPARs). Many such compounds are under intense development for the treatment of obesity and diabetes. There are currently two approved drugs in the class of thiazolidinediones – rosiglitazone and pioglitazone – that act on the PPARγ isoform of these receptors.

There is clear linkage between metabolic consequences of CR and activation of the fasting-responsive transcriptional coactivator, peroxisome proliferator-activated coactivator 1α (PGC-1α); however, sorting out the specific beneficial effects of the different PPAR agonists as CRM will require considerable research investment. Using gene transcript profiling in mice, Corton et al. (2004) made a strong case that manipulation of PPARα parallels that observed in CR. In vivo studies in nonhuman primate models of obesity and diabetes have also indicated the therapeutic potential of a PPARα activator. Results included reduced hyperinsulinemia and improvement of insulin-stimulated glucose uptake rate as well as lowered triglycerides and body weight (Schafer et al., 2004). Use of a PPARα activator also markedly improved lipid and insulin profiles in this primate model (Oliver et al., 2001).

PPARγ activators can also increase sensitivity to insulin, but recent studies have begun to question the rationale for this strategy. Rather than activating this pathway, a more effective strategy for treating metabolic disorders might be to modulate PPARγ activity to improve glucose homeostasis while preventing adipogenesis (Argmann et al., 2005). A connection between SIRT1 and PPAR has been made because SIRT1 represses PPARγ transactivation and inhibits lipid accumulation in adipocytes (Picard & Guarente, 2005). It has been hypothesized that reducing adipose tissue could have beneficial effects on lifespan related to the production of adipokines acting on target tissues, due to the indirect prevention of age-related metabolic disorders including diabetes or atherosclerosis (Picard & Guarente, 2005).

Lipid targets

Pharmacological manipulation of lipids offers many additional targets for candidate CRM; however, concepts for intervention are currently much less developed than for other targets. For
example, similar to the effects of CR, use of hypolipidemic drugs has shown efficacy in removal of damaged cellular components through enhanced autophagy (Donati et al., 2004). On the other hand, higher circulating levels of certain lipids, such as long-chain fatty acids, during CR may serve as an important signal for generating effects on tumor suppression, and enhanced immune responses as well as protection against oxidative stress (Yu, 1994). Higher levels of ketone bodies have been shown to provide protection against neurotoxic insults (Veech, 2004); however, this response appears to occur under regimens of CR that involve intermittent feeding rather than a sustained reduction in caloric intake (Anson et al., 2003).

Manipulation of adipokines can also be considered a target for CRM. Following the discovery of leptin, there was great optimism that manipulation of this adipokine would be a highly effective target that could regulate appetite and energy utilization (Wolf, 1996). This potential has not been fulfilled because of the apparent development of leptin resistance with long-term treatment. Nonetheless, a reduction in the level of circulating leptin observed in CR has been suggested to be the critical neuroendocrine modulator of the antiaging effects induced by this intervention (Shimokawa & Higami, 1999). To overcome the issue of leptin resistance, leptin gene transfer directly into the hypothalamus has been considered as a strategy (Kalra & Kalra, 2005). A single injection of a recombinant adeno-associated virus encoding the leptin gene can result in long-term reductions in fat, insulin, tryglycerides and free fatty acids (Kalra & Kalra, 2005).

Manipulation of adiponectin as a CRM offers another potentially important strategy. Levels of this adipokine are elevated in CR rats (Zhu et al., 2004). Circulating levels of adiponectin appear to be predictive of insulin responses to different diets in humans. Specifically, low levels of adiponectin correlate with reduced insulin sensitivity when nonobese subjects are placed on a short-term high-fat diet (Thamer et al., 2004). Injections of adiponectin in mice produces weight loss and reduced levels of serum glucose and lipids (Qi et al., 2004). Production of higher levels of adiponectin through gene therapy techniques has been shown to be effective in achieving reduced body weight and higher peripheral insulin sensitivity (Shklyave et al., 2003).

**Development of screens**

It is clear that the search for effective CRM can include a wide variety of targets. This search will be aided greatly by the development of a screening process to identify potential candidates. Screening processes can utilize simple cell systems or possibly simple invertebrate model systems, such as yeast. Targeting a cellular response, such as sirtuin activation, has already proven effective (Sinclair, 2005). Other targets, such as enhanced stress responses or even survival from stress, would likely also be useful. In these systems, the candidate CRM would be added directly to the model systems. Candidates emerging from such screens could then be evaluated in higher organisms, such as nematodes or fruit flies, to assess response to in vivo stressors, such as heat or hydrogen peroxide, and also to quantitate survival as an endpoint.

The recent development in our laboratory of an in vitro model of CR can also help accelerate development of CRM (de Cabo et al., 2003). A candidate CRM can first be administered to rats and mice over a short duration, e.g. 4 weeks. Then blood can be withdrawn and serum prepared and added to an in vitro cell system that is then subjected to a stress, such as heat or hydrogen peroxide. Using this assay, it has been shown that cells treated with serum derived from CR rats and monkeys exhibit enhanced survival after heat or oxidative stress (de Cabo et al., 2003). Importantly, serum from CR animals was also capable of inducing Sir2 expression in in vitro cell systems (Cohen et al., 2004). Thus, the rationale would be that serum derived from rats or mice treated with a candidate CRM should provide cells grown in vitro with greater protection against stress.

Beyond the in vitro screens, additional evaluation of in vivo effects will be needed. As we have argued, reduced body temperature and plasma insulin levels remain two important biomarkers of CR. Other hormonal responses should also be examined, including glucocorticoids, thyroid hormones, and adipokines, such as leptin and adiponectin. Another important in vivo screen that should be considered is effects of a candidate CRM on tumor growth in experimental models.

For both in vitro and in vivo screens, DNA microarrays can also be a highly useful strategy for developing CRM. The objective would be to determine if a candidate CR mimetic produced in a particular tissue produced a gene expression profile that was similar to that produced by CR (Dhahbi et al., 2005; Weindruch et al., 2001).

Ultimately, any candidate CRM would still need to be validated by demonstrating its beneficial effects on mortality, morbidity, and function in a relevant animal model, most likely a rodent model. Of course, this stage of development would be the most time-consuming and expensive component in the process. To assist in the discovery process, the National Institute on Aging has organized the Interventions Testing Program (Warner et al., 2000; National Institute on Aging Website, 2005). Acceptance into this program would allow an investigator to move a candidate CRM into a mammalian model and would assure quality control of the assessments.

**Conclusions and future directions**

Further evidence of the increased interest in the development of CRM can be found in Table 1. The websites of the biotech companies presented there now list CRM as one their primary development projects. High throughput in vitro screens established by such commercial enterprises will clearly accelerate the identification of candidate CRM.

As outlined in this review, many logical targets exist for assessing the potential effectiveness of these candidates. Use of different in vivo model systems will be applied to move these candidates forward. As candidates undergo different validation
assessments, the ultimate objective would be to evaluate a CRM in human trials. Because the acceptance of biomarkers of aging has still not been realized within the scientific community and, more importantly, within the regulatory arena, it is likely that disease endpoints will be used to assess the effectiveness of the candidates. Endpoints could include assessment of risk factors for heart disease and diabetes and for treatment or prevention of cancer, arthritis, stroke, and neurodegenerative disorders, such as Alzheimer and Parkinson diseases.

Another concept that will likely guide further development of CRM is that combinations of compounds could be more effective than single compounds (Everitt et al., 2005; Roth et al., 2005). This ‘cocktail’ approach may emerge in recognition of two key issues. First, because energy regulation is so key to an organism’s survival, there are likely to be compensatory mechanisms activated in response to manipulating a single target; thus, an intervention aimed at multiple targets could offset these compensatory responses. Second, effects of aging and the antiaging effects of CR involve multiple pathways; thus, interventions intended to provide protection to multiple systems and multiple tissues should be inherently more beneficial.

In conclusion, this emerging field appears poised for major advances. There will be many new concepts developed, many new approaches suggested, and many new candidate CRM identified as interest in this concept grows and matures.

References


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